Diazinon and Chlorpyrifos in the Central Contra Costa Sanitary District Sewer System, Summer 1996

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BACKGROUND

Historically, agricultural activities have been the focus of
investigations into pesticide impacts on water bodies. In recent
years, however, pesticide use in urban areas is increasingly
being examined as a potential source of aquatic pollutants.
Although applications of pesticides in urban areas are typically
on a small scale, the wide variety of chemicals used and the
frequency of applications can result in a substantial amount of
pesticides used. Urban-use pesticides can move off application
sites and enter storm drains which route surface runoffs into
urban creeks. These pesticides can also end up in urban sewage
which then travels to wastewater treatment plants.

Although conventional wastewater treatment techniques employed by
Publicly Owned Treatment Works (POTWs) may remove certain
pesticides with high efficiency, others may not be sufficiently
removed. Thus, these pesticides can be present in the treated
effluent and eventually be released into a receiving water body.
Under the California Porter Cologne Act, the Regional Water
Quality Control Boards (RWQCBs) regulate the quality of treated
effluent by issuing wastewater discharge permits to POTWs. These
permits prohibit toxic substances in the treated effluent at
concentrations that may cause harm to aquatic species.

In 1986, the San Francisco Bay RWQCB asked the Central Contra
Costa Sanitary District (CCCSD) in Martinez, California, to
initiate an Effluent Toxicity Characterization Program. The aim
of this program was to help characterize the toxicity of CCCSD's
treated effluent on selected aquatic test species. Twelve of the
18 tests performed under this program revealed that the treated
effluent was acutely toxic to the water flea, *Ceriodaphnia dubia.*
The RWQCB then requested that CCCSD perform toxicity
identification evaluation (TIE) studies to determine the cause of
the toxicity. The TIE studies suggested that two organophosphate
insecticides, diazinon and chlorpyrifos, were responsible for the
toxicity in CCCSD's effluent. Diazinon and chlorpyrifos are
commonly found in consumer and commercial products marketed for
urban uses. These uses are generally related to lawn/garden

care, indoor pest control, and pet care products.

PURPOSE

This study was jointly conducted by CCCSD and the Department of Pesticide Regulation to: 1) characterize the mean daily diazinon and chlorpyrifos concentrations and flow in the sewage of residential areas, selected commercial sites, and CCCSD treatment plant influent; 2) determine the relative importance of residential and commercial sources to the total influent load; and 3) compare the mean daily concentration and mass of CCCSD's influent to those of two other POTWs.

STUDY METHODS

Source sampling efforts were focused on the residential sector (single-family and multi-family residences) which contributes about 82% to CCCSD's influent load, and the commercial sector which contributes about 6%. Five residential areas were sampled daily in conjunction with daily sampling of CCCSD's influent for a one-week period. The areas contained as few as 829 and as many as 2,079 residences. Residential sampling occurred July 9-15, 1996.

Twelve commercial sites in the CCCSD service area (consisting of pet groomers, kennels, and pest control businesses) were also sampled. These three business types were selected because reconnaisance sampling by CCCSD had shown notable amounts of diazinon and/or chlorpyrifos in their effluent. Although sewage from other business types such as nurseries, restaurants, waste management facilities, and industrial facilities may also contain varying amounts of the two active ingredients, limited resources excluded their investigation. Unannounced sampling of selected commercial sources was done from July 18 through September 8, 1996.

The CCCSD influent samples were taken on a semi-weekly basis from June 22 through September 10; daily sampling occurred during July 9-16, August 4-11, and August 31 through September 7, 1996. For one week of the study, influent samples were collected from two other treatment plants in an effort to compare diazinon and chlorpyrifos concentrations among the POTWs: Union Sanitary District (USD) in Alameda County, and the Palo Alto Regional Water Quality Control Plant (RWQCP) in Santa Clara County. Simultaneous daily sampling of the USD and RWQCP occurred from August 5-11, in conjunction with the daily sampling of CCCSD.

The general sampling period was chosen so that warmer months

would be included. Insect problems and subsequent urban organophosphate use were expected to be greater during this period. Sewage samples were collected using programmed automatic samplers. For residential and commercial sampling, automatic samplers were suspended underneath manhole covers during operating periods and filled with blue-ice packs. Influent samplers were refrigerated units that were housed at the point of sewage entry into the treatment plants. All of the samples analyzed were flow-proportionally cornposited samples.

Representative flow data for residential, commercial, and influent sampling sites were also collected to allow the estimation of mass loads. Residential and CCCSD influent flow data were generated by CCCSD's flow modeling program known as the Sewer Network Analysis Program (SNAP). Commercial, USD influent, and RWQCP influent flow data were collected using inline flowmeters.

RESULTS

Influent Sampling:

Diazinon and chlorpyrifos were detected (reporting limit = 50 parts per trillion or ng/L) in all 37 of CCCSD's wastewater influent samples during the sampling period. The mean concentrations (calculated as the Uniformly Minimum Variance Unbiased estimator) of influent diazinon and chlorpyrifos were 310 ppt (parts per trillion) and 190 ppt, respectively. Influent diazinon concentrations ranged from 103 to 940 ppt. There are no diazinon and chlorpyrifos compliance criteria for treatment plant influent and other raw sewage concentrations. The treatment plant effluent; however, has to meet the "no toxicity" criteria enforced by the RWQCB.

Residential Sampling:

Residential sampling for diazinon and chlorpyrifos from five neighborhoods over a seven-day period (July 9-15, 1996) yielded 35 samples. The mean daily diazinon concentrations for each neighborhood were 740, 420, 120, 110, and 340 ppt. For chlorpyrifos, the mean daily concentrations for the same neighborhoods were 550, 110, 80, 110, and 180 ppt. Residential area diazinon concentrations ranged from none-detected to 4,300 ppt. Residential area chlorpyrifos concentrations ranged from none-detected to 1,200 ppt. The CCCSD service area's total daily residential mass loads for diazinon and chlorpyrifos were projected from sampling results to be approximately 42 g and 24 g, respectively.

Commercial Sampling:

Sampling of selected commercial businesses within the CCCSD occurred from July 18 through September 8, 1996. Of the 12 sites monitored, both diazinon and chlorpyrifos were found at seven sites. At two sites, only diazinon was detected. At two other sites, only chlorpyrifos was detected. Neither active ingredients were detected at the remaining commercial site. Daily concentrations of diazinon and chlorpyrifos were also variable. The highest level of diazinon (20,000 ppt) was found in the sewage from a kennel. The highest chlorpyrifos level (38,000 ppt) was found in the sewage from a pet groomer. Diazinon was detected on 17 out of the 32 days sampled. Chlorpyrifos was detected on 23 out of the 32 days sampled. CCCSD service area's total pet groomers, kennels, and pest control operators daily mass loads for diazinon and chlorpyrifos were projected from sampling results to be approximately 2.2 g and 2.3 g, respectively.

Concurrent POTW Sampling:

Concurrent sampling of the CCCSD, USD, and the RWQCP showed that the mean daily influent diazinon concentrations for the three POTWs were 300, 230, and 150 ppt, respectively. For chlorpyrifos, the mean daily influent concentrations were 190, 230, and 110 ppt, in the same order. Influent diazinon concentrations for CCCSD, USD, and RWQCP ranged from 130 to 750 ppt, 91 to 530 ppt, and 66 to 240 ppt, respectively.   Influent chlorpyrifos concentrations ranged from 140 to 230 ppt, 130 to 330 ppt, and none-detected to 150 ppt, in the same order. The only statistically significant finding among the POTWs was that the median influent chlorpyrifos concentration at RWQCP was less than those of CCCSD and USD.

CONCLUSIONS

Mass balance estimates revealed that residential sewage contributed the majority of the diazinon and chlorpyrifos to CCCSD's influent. Although much higher concentrations were occasionally seen in the sewage of selected commercial sources, the larger residential area flows translated to a greater residential contribution. Therefore, a source reduction strategy that focuses on reducing diazinon and chlorpyrifos loads from residential sources would be the most effective strategy. If such a source reduction program can successfully increase the pollution prevention awareness of service area residents, input from commercial and unknown sources may also decrease. The reduction of diazinon and chlorpyrifos influent loads will likely result in increased compliance with waste discharge permits.

ABSTRACT

This joint Department of Pesticide Regulation/Central Contra Costa Sanitary District (CCCSD) study characterized the mean daily diazinon and chlorpyrifos concentrations and flow of sewage from residential areas, selected commercial businesses, and CCCSD treatment plant influent. CCCSD influent was sampled semi-weekly for a 12-week period from 6/22-9/10/96. Sampling increased to daily during the periods of 7/9-7/16, 8/4-8/11, 8/31-9/7. Diazinon and chlorpyrifos were detected in all 37 influent samples taken at CCCSD. Influent samples from two other Publicly-Owned Treatment Works in Alameda County and in Santa Clara County collected from 8/5-8/11 showed that diazinon and chlorpyrifos were both detectable in 13 of 14 samples. The mean daily influent concentrations of diazinon and chlorpyrifos (as estimated by the uniformly minimum variance unbiased estimator) at CCCSD were 3 10 and 190 ng/L, respectively. Five residential areas in the CCCSD service area were sampled daily from 7/9-7/15. The mean daily diazinon concentrations for the five residential areas were 740,420, 120, 110, and 340 ng/L. The mean daily chlorpyrifos concentrations for the same areas were 550, 110, 80, 110, and 180 ng/L. Twelve selected commercial sites in the CCCSD service area (which included pet groomers, kennels, and pest control operators) were sampled individually throughout the period of 7/18-9/8. Diazinon and chlorpyrifos concentrations in commercial sewage were quite variable from one site to another. Samples with the highest diazinon and chlorpyrifos concentrations in this study (20,000 and 38,000 ng/L, respectively) were from commercial sources. Estimates of the percent contributions from residential and selected commercial mass loads to the CCCSD influent showed that residential sewage represented the majority of diazinon and chlorpyrifos source in the CCCSD collection system. Although much higher concentrations were occasionally found in the sewage of selected commercial sources, the larger residential area flows resulted in a greater residential contribution.

ACKNOWLEDGMENTS

DISCLAIMER

The mention of commercial products, their source, or use in connection with material reported herein is not to be construed as an actual or implied endorsement of such product.

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INTRODUCTION

In the recent past, the transport potential of pesticides from urban areas into aquatic ecosystems has been gaining recognition. Although individual applications of pesticides in urban areas are typically on a small scale, the wide variety of chemicals used and the frequency of applications made overall can make an urban area a notable source of pesticide contaminants (Kroll and Murphy, 1994; Immerman and Drummond, 1985).

Urban-use pesticides can move off application sites and enter storm drains which route surface runoffs into urban creeks (Schueler, 1995). In California, urban storm runoff is regulated by the State Water Resources Control Board (SWRCB) and the nine Regional Water Quality Control Boards (RWQCBs) through the issuance and enforcement of storm water discharge permits.

Urban-use pesticides can also end up in wastewater which makes its way via sewer lines to wastewater treatment plants. Thus, Publicly Owned Treatment Works (POTWs) are increasingly being scrutinized as another potentially significant contributor of these pesticides (Norberg-King et al., 1989; Ciba-Geigy, 1996). Single species toxicity testing (SSTT) and subsequent toxicity identification evaluation (TIE) of treated effluents of many POTWs have suggested that toxicity to test organisms such as Ceriodaphnia is associated with pesticides. Although conventional treatment techniques employed by POTWs may remove certain pesticides from wastewater with high efficiency, some may not be sufficiently removed. Thus, these pesticides can be present in the treated effluent and eventually be released into a receiving water body.

Under the Porter-Cologne Act, the RWQCBs issue wastewater discharge permits to POTWs. The permits prohibit toxic substances to be present in the treated effluent at concentrations that may potentially cause harm to aquatic species. As part of their waste discharge permit, POTWs can be required to initiate an Effluent Toxicity Characterization Program to characterize the toxicity of their treated effluent on selected aquatic test species. In 1986, the San Francisco Bay Regional Water Quality Control Board made such a request to the Central Contra Costa Sanitary District (CCCSD), a POTW located in Martinez, California. Results from the program revealed that 12 of the 18 test

events produced acute toxicity to *Ceriodaphnia dubia* (AQUA-Science, 1992).   The Regional Board then requested that CCCSD perform TIE studies to determine the specific cause(s) of the toxicity. TIE studies revealed that diazinon and chlorpyrifos, two organophosphate insecticides, were the contaminants most likely to be causing toxicity in CCCSD's treated effluent (AQUA-Science, 1993). Consequently, CCCSD has been investigating sources of diazinon and chlorpyrifos in addition to providing educational outreach to its service area's population in hope of reducing pesticide discharges into the sewers.

Diazinon and chlorpyrifos are commonly found in consumer and commercial products marketed for urban uses. Such uses are generally related to lawn/garden care, indoor pest control and pet care products. Urban uses of these products can result in the introduction of these organophosphates into sewage. Samples previously taken from the CCCSD sewer system showed that diazinon and chlorpyrifos were present in wastewater from residences, commercial pesticide applicators, pet grooming businesses, and kennels (AQUA-Science, 1995).

In 1996, DPR and CCCSD jointly participated in a monitoring study focussing on the various sources of diazinon and chlorpyrifos in the CCCSD sewage collection system (Sanders, 1996). The objectives of this study were to 1) characterize the average daily concentrations and mass of diazinon and chlorpyrifos in sewage of residential areas, selected commercial businesses, and CCCSD treatment plant influent; 2) estimate the mass contributions of residential and selected commercial sources to the CCCSD influent; and 3) compare the average daily concentrations and mass of CCCSD's influent to those of two other nearby POTWs. Results will be used to help develop a feasible source control strategy for diazinon and chlorpyrifos in the CCCSD service area.

MATERIALS AND METHODS

<u>Study Area</u>

Figure 1 shows the general location of the three POTWs involved in this study. The primary study area was the service area of CCCSD's sewage collection system (Figure 2). The service area covers

Figure 1: The Locations of Participating POTWs in the San Francisco Bay Area.
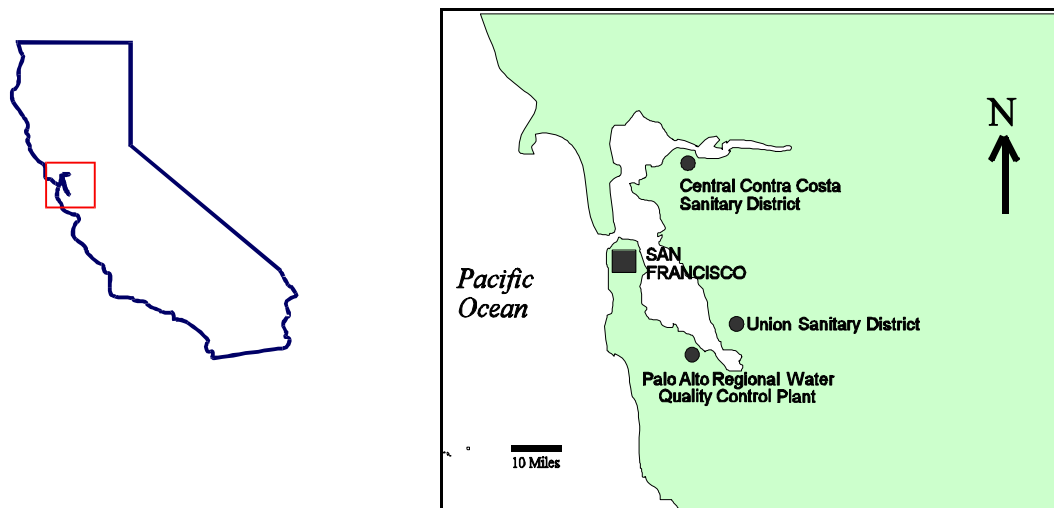
Figure 2: The Central Contra Costa Sanitary District (CCCSD) Service Area. Residential sampling areas are also shown.
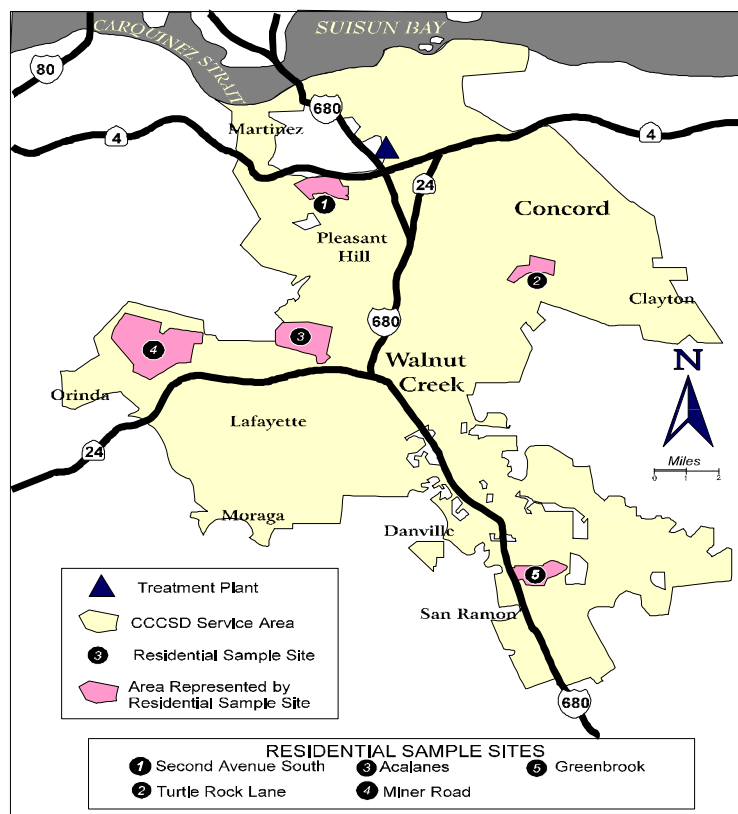
a large portion of Contra Costa County serving nearly 400,000 residents in 10 cities (including Concord and Walnut Creek) and two unincorporated towns. The CCCSD treatment facility is located in Martinez adjacent to Suisun Bay where the facility's treated effluent is released.

For one week of the study, influent samples were also collected from two other area treatment plants: Union Sanitary District (USD) in Alameda County and the Palo Alto Regional Water Quality Control Plant (RWQCP) in Santa Clara County. USD serves approximately 286,000 residents in the cities of Fremont, Newark, and Union City. RWQCP serves 200,000 residents in the cities of East Palo Alto, Los Altos, Los Altos Hills, Mountain View, Palo Alto, and Stanford University.

Sampling Overview

CCCSD's treatment plant influent was sampled to determine the average daily concentrations of diazinon and chlorpyrifos. Selected residential areas and commercial sites in the CCCSD collection system were also sampled for the two pesticides. Flow data associated with these monitoring points enabled the daily mass loads to be determined. The residential and commercial mass values were then used to project the total service area's residential and commercial mass contribution to the treatment plant influent.

The influent at USD and RWQCP were sampled concurrently with CCCSD's influent for one week to compare diazinon and chlorpyrifos concentrations among the three POTWs. Influent flow data from each POTW were used to estimate the mass of the two active ingredients entering the treatment plants.

Sampling Strategy and Schedule

Within the CCCSD service area, many source classifications contribute to the treatment plant influent. Table 1 shows the flow contributions of various sources to the CCCSD influent. The residential portion (single family and multi family residences) accounts for about 82% of the influent flow. The next largest source is that of the commercial sector which comprises about 6% of the

influent flow. Six other sources make up the remaining 12% of the influent. To keep monitoring efforts manageable, sample collection was concentrated on selected residential and commercial sources in addition to the plant influent. DPR/CCCSD attempted to locate sampling points that best isolated the target source. However, samples taken from targeted residential areas or commercial sites may have contained small amounts of sewage from non-target sources (e.g. offices, shops, churches).

Table 1: Flow Contributions of Various CCCSD Sources to the Influent (data from CCCSD)**.**

Source Classification	July 1996 Flow Estimates (gal/day)
Single Family Residences (SFR)	23,100,000
Multi-Family Residences (MFR)	8,500,000
Commercial	2,300,000
Industrial	1,700,000
Office	1,100,000
Point Source	1,000,000
Schools	600,000
Churches	100,000
Total	38,400,000

A point source is an industrial/commercial establishment with a high use of water per acre occupied.

Residential areas were selected based on the following criteria: 1) no commercial or industrial dischargers were in the area, 2) the presence of sampling sites that were accessible, 3) sites allowed for accurate flow metering, 4) they provided a representative socio-economic cross-section of the community, and 5) demographic data of the areas were available from a CCCSD residential metal study (Larry Walker & Associates, 1993). Collectively, R01-R05 comprise nearly 5% of the service area's residential flow. Information on the selected areas are listed in Table 2.

Table 2: Residential Sampling Areas (data provided by CCCSD).

Area #	Sampling point	General Location	Residences per Area	Acreage per Area	Density (residences/acre)
R01	2nd Ave. South	Martinez/Pacheco	2,079	684	3.0
R02	Turtle Rock Lane	Concord	1,593	313	5.1
R03	Acalanes Road	Lafayette	829	773	1.1
R04	Miner Road	Orinda	858	1,285	0.7
R05	Greenbrook Court	Danville	991	345	2.9

Twelve commercial sites in the CCCSD service area were selected for sampling. For confidentiality, the names of target businesses will be omitted from this report. Instead, they will be simply referred to as sites C06-C17. In this study, commercial sites were generally classified into three groups: pet groomers (C06-C10), kennels(C11-C12), and pest control operators (C13-C17). Detections of diazinon and chlorpyrifos in past CCCSD source surveys justified the selection of these three groups (AQUA-Science, 1995). Specific commercial sites were chosen on the basis that 1) no other potential sources or users of pesticides were located upstream from the site, 2) sites were accessible and allowed for accurate flow measurement, 3) sites represented a variety of geographic cross-sections of the CCCSD service area, and 4) these locations were adequately safe for the sampling crew.

The sampling period was selected so that warmer months would be included (Table 3). Insecticidal activities and subsequent urban organophosphate use were expected to be high during this period. Efforts to concurrently take comparative samples for mass loading purposes were made such as the first phase of residential sampling (week 4) and POTW sampling (week 8). The frequency of CCCSD influent sampling increased to daily during these weeks. Commercial sampling was scheduled in between more intensive sampling periods to fit sampling crew availability and alleviate laboratory load.

Table 3: Weekly Sampling Schedule by Sample Types

Sample Type	Week 1 6/19-23	Week 2 6/24-30	Week 3 7/1-7	Week 4 7/8-16	Week 5 7/17-21	Week 6 7/22-28	Week 7 7/29-8/4	Week 8 8/5-12	Week 9 8/13-18	Week 10 8/19-25	Week 11 8/26-9/1	Week 12 9/2-8	Week 13 9/9-11	Total
CCCSD Influent[1]	2*	2	1	7	2*	2*	2	7*	2	1	2	7	1	**38**
Union San Influent								7						**7**
Palo Alto Influent[2]								14						**14**
Residential (5 sites)[3]	1*			35					8					**44**
Commercial (12 sites)[4]	2*				3	5	6	2	4	4	3	3	1	**33**
Field Blanks	1*			5	1*	2	2	5*	3	2	2	1		**24**
Equipment Blanks	1*		1	1		2*	1		1		1			**8**
Blind Spikes[5]	2						2	2		2				**8**
Total	**7**	**2**	**2**	**48**	**6**	**11**	**13**	**37**	**18**	**9**	**8**	**11**	**2**	**176**

[1] = Two influent samples were collected each week, normally on Sunday and Wednesday. During weeks 4, 8 and 12, daily samples were taken for 7 days. All influent samples after week one were 24-hr. composites.

[2] = Two samples per day, 14 total, instead of seven are necessary at the Palo Alto plant because the pesticide contribution of the plant's recycled water needed to be distinguished from the influent contribution.

[3] = Residential samples from week 1 were grab samples for split sample comparison. Week 4 of residential sampling consisted of simultaneous 24-hr. composites at five sites. Week 9 is comprised of 3-hr. composites for a 24 hr. period at one site.

[4] = Commercial samples from week 1 were grab samples (kennel) for a split sample comparison. The remaining commercial samples were 24-hr. composites. One to three composite samples were taken from each commercial site.

[5] = Blind spikes were prepared by a DPR-approved laboratory and sent to APPL for analysis as an additional laboratory QC measure.

* = Split sample comparison: GC/NPD by APPL, GC/MS by CCCSD, Ciba-Geigy and Dow-Elanco, and ELISA by AQUA-Science (see Appendix E).

Although the schedule was initially developed to make optimum use of available resources, occasional samples were lost due to obstruction of suction tubes, insufficient volume of sample drawn, or errors in programming of the autosampler. For example, several commercial sites did not produce the scheduled three daily samples because of autosampler failure. If possible, sampling was rescheduled.

Sampling and Flow Measurement Methods

CCCSD Influent - CCCSD Influent samples were taken from the sewage entry point immediately after the debris screens. A Sigma® model 900 refrigerated-autosampler connected to the plant's influent flowmeter was programmed to take flow-proportioned samples. Thus, for a predetermined volume that entered the plant (based on historical daily flow), the autosampler collected a fixed volume in response. The influent subsamples were pumped into a 9.5-L glass jar housed in the autosampler unit that maintains a temperature of 3EC. At the end of the 24-hour sampling period, the composite jar was capped and taken to CCCSD's on-site field operations facility (Bay 11) where the sample was processed. Field personnel swirled the sample for 30 seconds. Approximately 500 ml of the sample were decanted through a clean glass funnel into a 1-liter amber glass bottle. Swirling resumed for another 10 seconds after which another 500 ml were decanted into the bottle. The finished sample was sealed with a Teflon®-lined cap and refrigerated until it was packaged for shipping.

All 1-L amber glass sample bottles, caps, and external Tygon® tubing for the autosamplers were new and were used for a single event or site. All other equipment and glassware were washed using Alconox® soap and water with a deionized water rinse between uses.

Semi-weekly sampling (Sunday and Wednesday morning retrievals) of the CCCSD influent was conducted for a 12-week period. In addition, daily sampling occurred during July 10-16, August 5-11 and September 1-7, 1996.

CCCSD Residential Areas - In the first phase of residential sampling, seven daily samples were

taken concurrently from the five residential areas. Sewage subsamples were drawn using ISCO®
2700, 2900 and 3700 autosamplers stocked with 24 (350 ml), 12 (375 ml), and 12 (375 ml) glass
bottles, respectively. The ISCO® 2700 collected 85 ml-subsamples every 15 minutes. The ISCO®
2900 and 3700 autosamplers collected 90 ml-subsamples every 30 minutes. Field crew suspended
the battery-operated autosampler unit from the lip of the manhole underneath the manhole cover.
Sewage samples were drawn from the waste stream by the autosampler through a Tygon®-suction
tube equipped with a debris guard.

During sample retrieval, the autosampler was raised out of the manhole with a motorized winch.
Field crew checked and cleaned the intake tube and debris guard of obstructions. The sample bottles
were removed, capped, and placed in coolers with wet ice for transport. The autosampler was
restocked with clean bottles and frozen blue ice. Finally, field personnel reprogrammed the
autosampler and placed the unit back in the suspended position for the next sampling period. The
subsamples were taken to CCCSD's Bay 11 where they manually flow-composited into a 1-L amber
glass bottle with a Teflon®-lined cap. The resulting composite samples were then refrigerated prior
to shipment to the laboratory. The first phase of residential sampling occurred from July 9-15, 1996.

Hourly flow data used for the flow-proportioned compositing for each site were compiled for a
weekday and a weekend 24-hour period prior to sampling by CCCSD personnel. Flow data were
obtained using the Montedoro-Whitney® flowmeter (with a Q-Logger and Sonic Star depth/velocity
probe). Manual compositing of the sewage subsamples was achieved by determining the ratio of the
hourly flow to the total daily flow at each site. For example, if 20% of the day's flow at R05
occurred between 7 a.m. and 8 a.m., then 200 ml from the bottle(s) which sampled that period would
be composited into the 1-L amber bottle. Subsamples for compositing were shaken for 30 seconds
and poured through a glass funnel into the bottle. The flow at each residential site was monitored for
one day during the sampling week to assure accuracy to the previous flow characterization data.

Flow measurement checks indicated that the hourly flow proportions remained fairly constant,

however, total daily flow volumes varied from previous measurements at several sites. These discrepancies suggested that the flowmeter used may not have been operating properly. Since erroneous flow values would have affected subsequent mass load calculations, alternate residential flow estimates were obtained from the CCCSD's Sewer Network Analysis Program (SNAP).

The SNAP modeling program uses data on various land use-types (i.e. schools, SFR and MFR), infiltration, and influent flow to determine an area's flow volume. CCCSD has determined that SNAP estimates conform with historic flow monitoring results obtained from the field (Lai, 1996). Since SNAP estimates were used for residential areas, the use of SNAP estimates for the treatment plant influent would keep the flow terms consistent on both sides of the mass balance equation. A comparison of the SNAP influent flow estimates to the flowmeter measurements during the residential sampling period showed only a one percent difference in volume.

The second phase of residential sampling was intended to give a concentration profile of diazinon and chlorpyrifos at a residential neighborhood in a one-day period. R02 was selected for second phase monitoring based on having had the most detections of diazinon and chlorpyrifos for the week of phase one monitoring as well as for logistical and safety considerations. Field personnel collected samples using an ISCO® 2700 with a 24-bottle configuration and 15-minute sample pacing. After retrieval, three one-hour subsamples were composited together resulting in 8 composite samples. Each one-hour subsample was shaken for 30 seconds. 333 ml of each subsample were measured in a graduated cylinder and decanted through a glass funnel into a labeled 1-L amber bottle and capped. Flow-proportioned compositing was not necessary because concentration results were not intended to produce mass values. The second phase was conducted from August 13-14, 1996.

CCCSD Commercial Sites - ISCO® 2900 autosamplers were used to monitor the commercial sites. The setup of the autosampler was similar to that of residential monitoring. However, an ISCO® Flow Poke (with a model 4230 bubbler meter) was connected to the autosampler, enabling collection of a real-time flow-proportioned composite sample. Samples were collected directly into a single 9.5-L composite glass jar. The flowmeter is designed to accurately measure flow in low-flow

conditions. Only one ISCO® Flow Poke meter was available; therefore the commercial sites had to be sampled sequentially. Commercial samples were processed in Bay 11 in the same manner as the influent samples.

Commercial site sampling was conducted from July 18 through September 8, 1996. The day of the week during which a particular commercial site was sampled depends on its expected peak business period. Pet groomers and kennels were monitored Friday through Sunday. Pest control operators (PCOs) were monitored on weekdays.

USD and RWQCP Influents - The influents of CCCSD, USD, and RWQCP were sampled concurrently to compare influent concentrations and mass loads. USD and RWQCP were equipped similarly to CCCSD with Sigma® model 900 refrigerated-autosamplers programmed to take flow-proportioned, composite samples. RWQCP recycles water used in the treatment process (e.g. water used to clean incinerator stack filters) back into the waste stream prior to the influent autosampler. Thus, this input had to be accounted for since it has a potential to contaminate the influent sample. The use of another Sigma® refrigerated-autosampler on the recycle stream allowed the recovery water to be sampled for diazinon and chlorpyrifos. Flowmeters at the influent provided the flow measurements for all three POTWs.

Simultaneous daily sampling of CCCSD, USD, and RWQCP occurred from August 5-11. DPR provided 1-L amber glass bottles, glass funnels, chain-of-custody forms, insulated coolers and blue ice to each POTW for the study. Sampling personnel for each POTW were given sample handling and processing procedures identical to those used at CCCSD.

Field Quality Control

Due to the low concentrations of the analytes expected in the samples, field blanks and equipment blanks were used for field quality control in this study. To help detect site-specific contamination, a field blank was collected at each site during sample collection. After being filled with deionized water at the site, blanks were subjected to the same treatment as the post-collection sewage samples.

Equipment blanks or rinse blanks were used to assess the cleanliness of the autosampler setup and were prepared in a sheltered location (e.g. CCCSD's Bay 11). These blanks mimicked a sewage sample from when it was taken to when it was deposited into the sample container. Deionized water was drawn by a clean autosampler through its debris guard, external and internal tubing, and deposited into a clean autosampler-glass bottle. This water was poured through a cleaned glass funnel into a new 1-L amber glass bottle and capped. Equipment blanks were done every 2 weeks to verify that equipment cleaning standards and protocols (Lescure, 1996) were adhered to.

Sample Handling

All samples received identical treatment after collection. Each 1-L amber glass bottle was filled to capacity to prevent trapping of air in the bottle. Since diazinon and chlorpyrifos can degrade under basic and acidic conditions, the pH of the samples was not adjusted from their typically neutral pH. Samples were then refrigerated at 4EC until they were ready to be shipped out. Samples were placed in insulated coolers with packing material to lessen the chance of breakage. Blue-ice packages were then added to the cooler to help maintain a temperature near 4EC. An overnight delivery service delivered the samples to the respective analytical laboratory for immediate extraction and subsequent analysis. Samples collected on Monday through Thursday were shipped the day they were collected. Samples collected from Friday through Sunday were shipped on the following Monday. The average time between sampling and laboratory extraction was about 5 days.

Chemical Analysis and Laboratory Quality Control

Analytical Method - Samples were analyzed for the presence of diazinon and chlorpyrifos by the Agriculture & Priority Pollutants Laboratories (APPL) Inc. located in Fresno, California. Sample extracts were prepared by solid phase extraction using the 90 mm C18 3M Empore™ extraction disk. If necessary, florisil cleanup was used following solid phase extraction. Extracts were analyzed by EPA method 8141A using a Hewlett Packard 5890 gas chromatograph equipped with 7673A

autosamplers or a Hewlett Packard 6890 gas chromatograph equipped with 6890 autosamplers. Dual columns were used for elution of the extracts. The first column was a 30-m x 0.53-mm wide-bore capillary column, 1.5-µm film thickness, chemically bonded with 35% phenyl methyl polysiloxane (HP-35). The second column was a 30-m x 0.53-mm wide-bore capillary column, 1.0-µm film thickness, chemically bonded with 5% phenyl polysiloxane, 95% methyl polysiloxane (DB-5). The detector used was a Nitrogen-Phosphorus Detector (NPD) operated in the phosphorus-specific mode. The complete APPL analytical method is provided in Appendix A.

Method Development - As required by the DPR Standard Operating Procedure QAQC.001.00 (Appendix B), APPL underwent a series of developmental steps to achieve the analytical methods for this study. The specific requirements are outlined in the Analytical Laboratory Specifications (Appendix C) and involved three primary stages: 1) the method detection limit study, 2) the analyte(s) degradation study and 3) the method validation study.

Laboratory Quality Control - In addition to the standard laboratory D.I. spikes and blanks conducted by APPL, matrix spikes and blanks were also performed with each extraction set as required by DPR SOP number QAQC.001.00. Blind in-house matrix spikes were also performed by APPL periodically. Moreover, several samples extracted by APPL were sent to the California Department of Food and Agriculture's (CDFA) Chemistry Laboratory Services in Sacramento, California for analysis as an additional quality control check. The results of the laboratory quality control data and a detail discussion of these results are presented in Appendix D.

Split Sample Comparison - Sewage from various source types (residential, commercial, influent, etc.) was split and sent to several participating laboratories for comparative analysis. The exercise was conducted to see if each laboratory could produce similar results in analyzing for diazinon and chlorpyrifos in a complex sewage matrix. Knowledge gained from the exercise will increase understanding in the comparability of future results, and possibly improve analytical methods.

Two rounds of split sample comparisons were completed during the course of the study. The first began in mid-June and consisted of seven samples. APPL, CCCSD, AQUA-Science and Dow-

Elanco analytical laboratories analyzed samples in this first round. The second round started in the end of July and continued into August and included 19 samples. In addition to the participating laboratories in the previous round, the Ciba-Geigy laboratory also participated in the second round. Appendix E contains a detailed discussion of the split sample comparison.

Data Analysis

All data used for analysis in this report have been adjusted for recovery from the raw results with the exception of split sample comparison data which were unadjusted (Appendix E). Laboratory quality control data showed that the first set of Empore™ extraction disks were responsible for low recoveries for both diazinon and chlorpyrifos (see Appendix D for details). Follow-up research by the disk manufacturer confirmed the disk's elution inefficiency when methylene chloride is used as the solvent. The replacement of these original disks with larger diameter disks improved recovery considerably. Since recoveries changed due to a modification of the analytical method during the study, all raw data reported by the laboratory were adjusted by sample batches to 100% recovery based on each batch's average matrix spike recovery.

Reporting limits were also recovery-adjusted on a batch basis to be consistent with treatments to analytical results. Prior to adjustments, the default APPL reporting limit for diazinon and chlorpyrifos is 50 ng/L. Subsequent adjustments may shift a batch's reporting limit above or below this value. Thus, the reporting limit may appear as for example <76 ng/L or <39 ng/L. The less than symbol prior to a numeric value denotes a reporting limit. Subsequent uses of the term "reporting limit" will not be accompanied by the clarification of "<50 ng/L" since it should be understood that this default limit would have shifted in value from batch to batch. Adjusted reporting limits for each analytical batch are shown in Appendix D, Tables D1&D2. To keep charts and graphs easy to interpret in this report, only results above the reporting limit will be plotted.

The distribution characteristics of the sampled population were determined prior to the use of any summary or descriptive statistics. Data analysis was accomplished using both MINITAB® (1994) and SAS® (1994) statistical software. Various statistical tests and procedures were used to help

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answer more specific questions pertaining to the data.

To establish a representative daily concentration value for the influent and residential data, the estimate of the true mean (a) of the untransformed, lognormally distributed concentration data was required. The simple back-transformation of the mean of the log-transformed data (sample geometric mean) may seem to be a suitable choice for the determination of a representative average; however, this technique was not used since it produces a mean estimate with a large negative bias (Parkhurst, 1998; Blackwood, 1992; Ung and Vegiard, 1988). Therefore, the geometric mean tends to underestimate the true mean of a lognormal distribution. For the purpose of calculating an unbiased estimate of the true mean (a), the more appropriate method in this case is the Uniformly Minimum Variance Unbiased (UMVU) estimator via the method of Bradu and Mundlak (1970). A SAS® program developed by Powell (1991) was employed in the calculation of all Bradu-Mundlak UMVU estimators in this study. In this report, the UMVU estimator will occasionally be referred to as "estimate of the true daily mean (a)" or simply as "a-estimate".

The residential concentrations for both diazinon and chlorpyrifos contained censored values (values below the reporting limit (RL)). These censored values had to be addressed before any analysis could proceed. Simple substitution for all values below the reporting limit (RL) was an option. However, inserting a constant (e.g. 0, ½ RL or the RL) for all values below the RL could lead to undesirable bias and errors in subsequent summary statistics (Slymen et al, 1994; Helsel, 1990; Helsel and Cohn, 1988). The treatment of censored values can have an impact on the resulting estimate. Thus, the robust probability plotting method for a single reporting limit was used to obtain "fill-in" values for the observations below the RL (Helsel, 1990). This method has the advantage that it does not assume any particular distribution to assign the fill-in values. Furthermore, the fill-in values are based only upon the data observed above the RL.

In order to estimate the diazinon and chlorpyrifos total daily mass load of the three POTWs' influents as well as CCCSD's commercial and residential sources, the most representative flow and concentration values were used. When possible, Land (1971) exact confidence intervals accompanied estimates to better describe calculated mass values.

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RESULTS & DISCUSSION

CCCSD Influent

From June 22 to September 10, 1996, 37 raw sewage samples were taken from CCCSD's influent and analyzed for diazinon and chlorpyrifos (Figure 3, on the following page). Diazinon and chlorpyrifos were detected in all of the influent samples. None of the field blanks associated with the CCCSD influent site contained detectable levels of contaminants. Raw and recovery-adjusted influent results are presented in Table F1 in Appendix F. All statistical analysis results presented are for recovery-adjusted data.

Both influent diazinon and chlorpyrifos concentrations failed the Shapiro-Wilk test (Shapiro and Wilk, 1965) for normality ($p = 0.01$ and $p = 0.01$, respectively). The log-transformed (natural logarithm) concentration data were subjected to the same test. The log-transformed diazinon concentrations did not fail the test for normality ($p = 0.10$), allowing the assumption that the influent concentrations were lognormally distributed. The log-transformed chlorpyrifos concentrations still exhibited significant departure from normality ($p = 0.01$). However, this departure from normality was due entirely to one particularly large occurrence of chlorpyrifos. Omitting this one observation, CCCSD's chlorpyrifos concentrations were also assumed to follow a lognormal distribution. With the assumption of lognormality, subsequent descriptive and test statistics used also reflect this distribution.

Figure 3: CCCSD Daily Influent Diazinon and Chlorpyrifos Concentrations (6/22-9/10/96)

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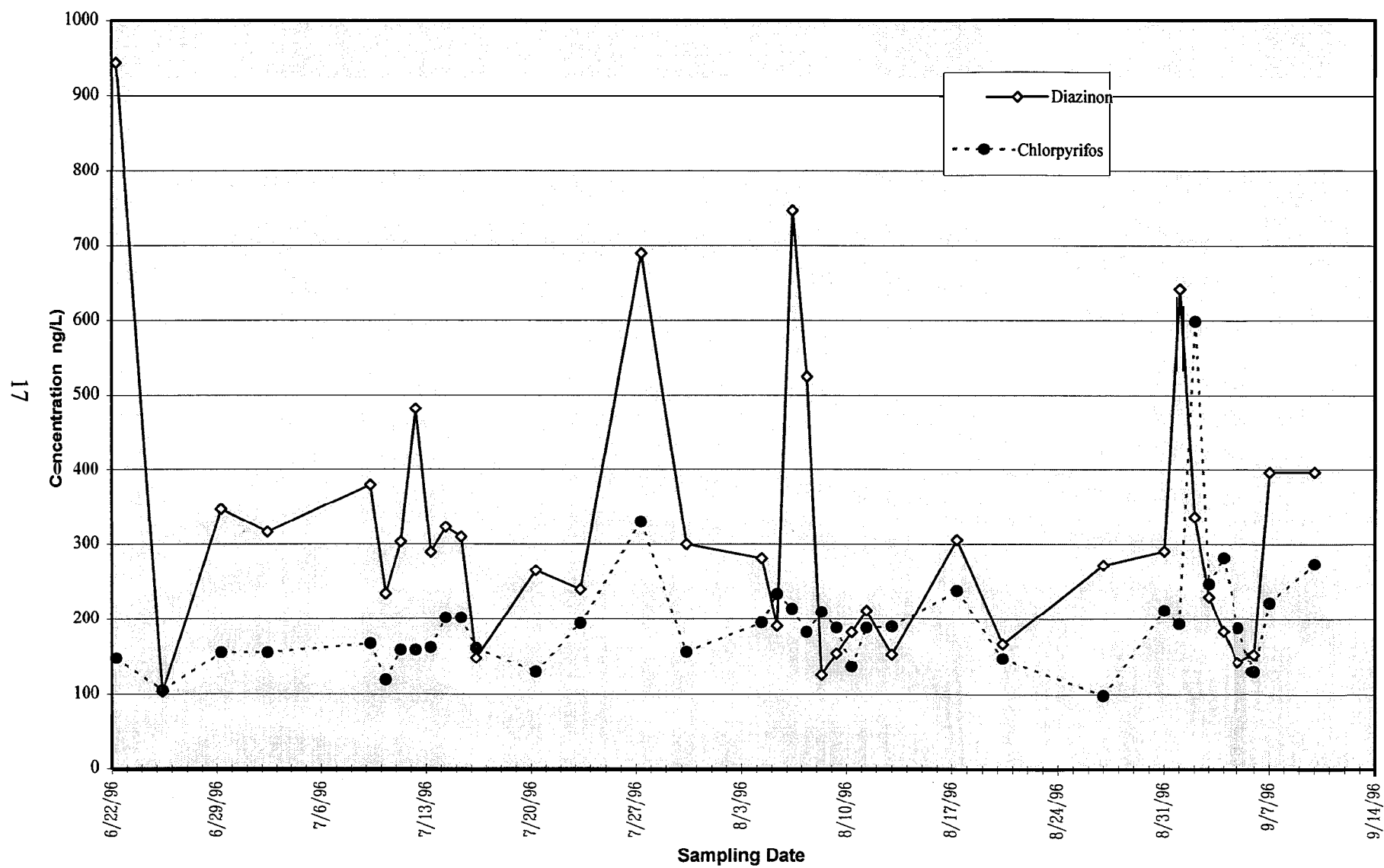
Of the 37 influent samples taken, 13 represented weekend samples. The remaining 24 influent samples were collected on weekdays. Plots of weekend versus weekday samples for both diazinon and chlorpyrifos are shown on Figures 4 & 5 (following page). Mann-Whitney tests were performed on the log-transformed weekend and weekday data for both diazinon and chlorpyrifos. The tests indicated that diazinon weekend concentrations were not significantly higher than the weekday concentrations (p = 0.47). Likewise, chlorpyrifos concentrations on the weekends were not significantly different from weekday concentrations (p = 0.05).

Figure 4: Comparison of Weekend and Weekday Influent Diazinon Concentrations

Figure 5: Comparison of Weekend and Weekday Influent Chlorpyrifos Concentrations

18
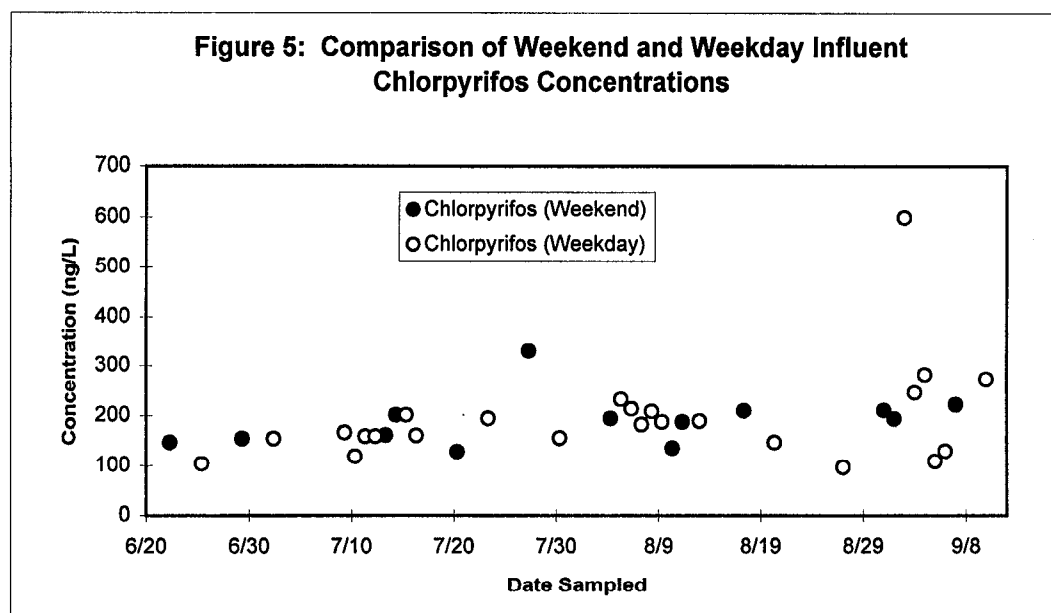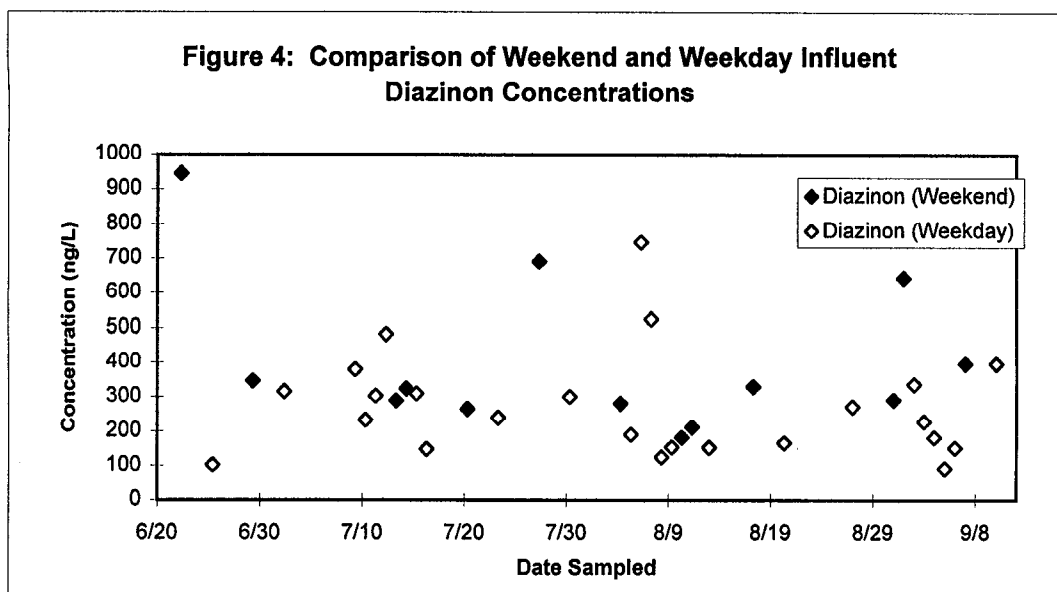
Based on the 37 influent samples, the true daily mean (a) influent diazinon concentration was estimated, using the UMVU estimator method, to be 310 ng/L. The true daily mean (a) influent chlorpyrifos concentration was estimated to be 190 ng/L (Table 4).

Table 4: UMVU Estimates of True Daily Mean (a) Influent Diazinon and Chlorpyrifos Concentrations

Influent Sampling Period	a-Estimate of Influent Diazinon Concentration (ng/L)	a-Estimate of Influent Chlorpyrifos Concentration (ng/L)
6/22/96 - 9/10/96	310	190

Influent diazinon concentrations ranged from 103 to 940 ng/L. The highest influent concentration of diazinon of 940 ng/L was measured on the first day of sampling. The concentration was approximately three times the influent diazinon a-estimate of 310 ng/L. The second to fifth highest diazinon detections were at 750, 690, 640, and 530 ng/L. The five highest diazinon concentrations were generally scattered over the influent sampling period. The lowest diazinon concentration of 103 ng/L occurred on Tuesday, June 25th. Thus, only a 3-day period separated the highest and lowest diazinon level during the study period. The five lowest diazinon concentrations were scattered over the sampling period.

For chlorpyrifos, influent concentrations ranged from 98 to 600 ng/L. The highest influent concentration of 600 ng/L was measured on Monday, September 2, or Labor Day. This peak chlorpyrifos detection was also about three times its corresponding influent chlorpyrifos a-estimate of 190 ng/L. The second to fifth highest chlorpyrifos detections were 330, 280, 270, and 250 ng/L. Four of the five highest concentrations were measured in early September. The lowest chlorpyrifos concentration of 98 ng/L occurred on Tuesday, August 27th. The five lowest chlorpyrifos concentrations were scattered over the sampling period. Insufficient influent data exist in this study to establish a time-series or seasonal trend.

Collection of 35 residential diazinon and chlorpyrifos samples coincided with the July 9-15 CCCSD influent sampling. Five neighborhoods (areas number R01-R05) within the collection system were the focus of the residential portion of the study. The sewage from these five neighborhoods was sampled from manholes on 2nd Avenue South (R01 in Martinez/Pacheco), Turtle Rock Lane (R02 in Concord), Acalanes Road (R03 in Lafayette), Miner Road (R04 in Orinda), and Greenbrook Court (R05 in Danville). Unlike the CCCSD influent, diazinon and chlorpyrifos were not always detected in the sewage samples. Residential monitoring results are presented in Figures 6 & 7 (on the following page). Note that the concentration scales on these two graphs are different. Field and equipment blanks associated with the residential sampling sites contained no detectable concentrations of either active ingredients.

The concentration data from these five neighborhoods were used to estimate the mass load values for all neighborhoods in the CCCSD service area. The censored data were not uniformly distributed among the five sites. However, all the data were pooled as one group to increase the sample size and to properly characterize the data set. This treatment was also necessary because R03 contained only two observations above the RL making this neighborhood unsuitable for individual characterization. The site of origin for each measurement was retained, and once the fill-in values were calculated, the data were re-sorted for analysis by site. The retention of neighborhood of origin was necessary because the sewage from each site is received separately at the treatment plant. Therefore, to obtain a daily average concentration for the overall residential flow of the CCCSD influent, the data were physically generated from a nested sampling design. The residential concentration data set with the fill-in values was used for all analysis. The raw, recovery-adjusted, and fill-in residential data are shown in Table F2 in Appendix F.

Initial analysis of residential diazinon and chlorpyrifos concentrations, using the Shapiro-Wilk Test indicated that they were not normally distributed ($p = 0.01$ for both diazinon and chlorpyrifos). These results were not surprising since the residential flow was a major component of the influent which was assumed to conform to a lognormal distribution. Subsequently, the residential

Figure 6: Daily Residential Diazinon Concentrations (7/9-7/15/96)

missing columns indicate results were below the reporting limit for that day.
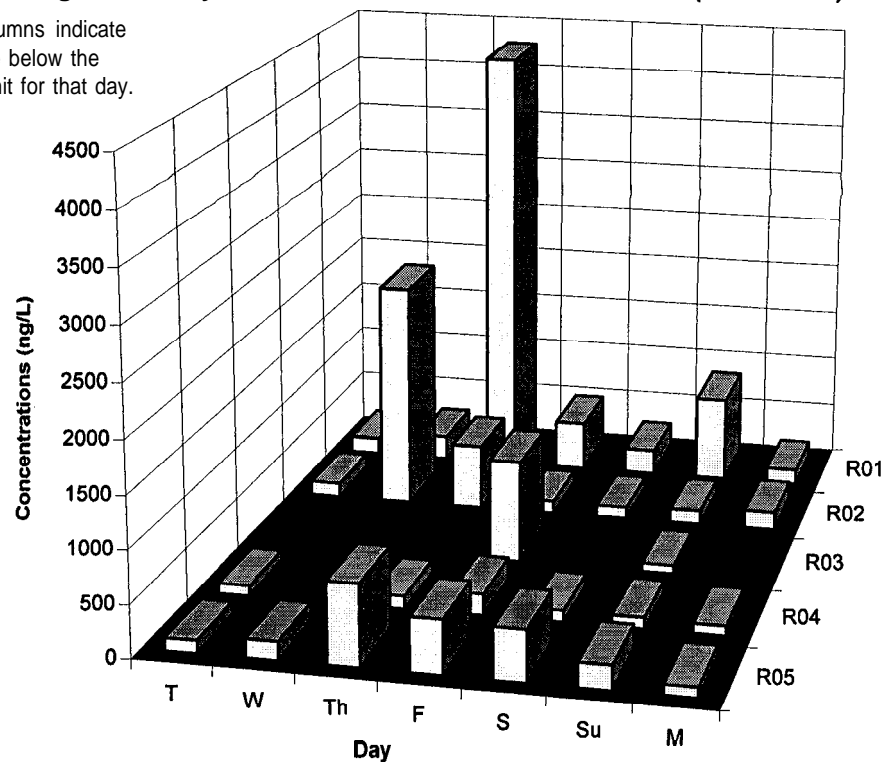
Figure 7: Daily Residential Chlorpyrifos Concentrations (7/9-7/15/96)

missing columns indicate results were below the reporting limit for that day.

21
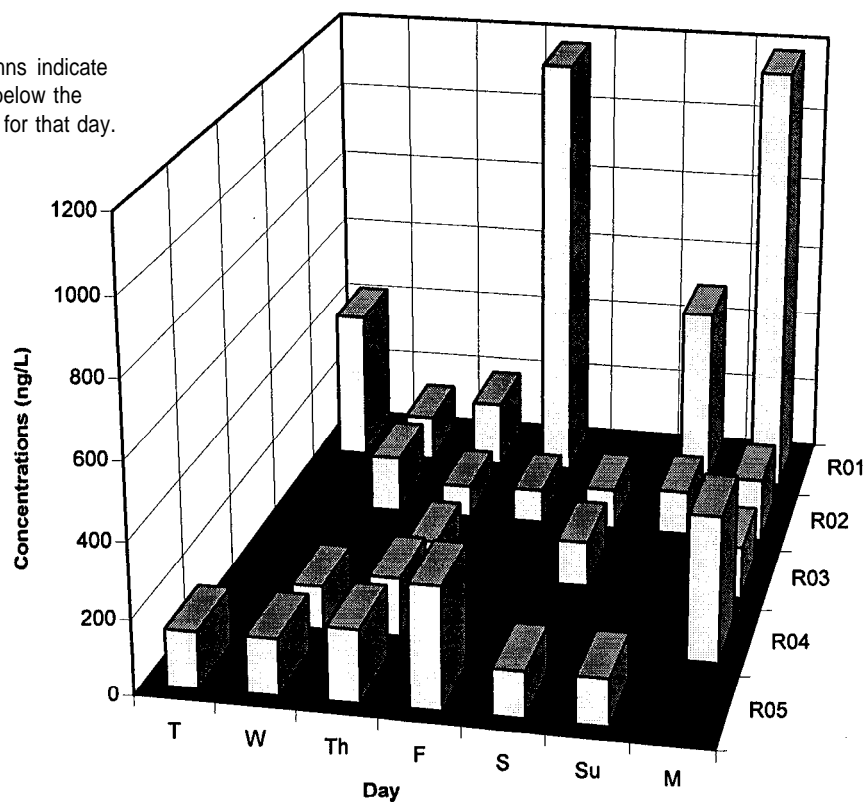
concentration data were log-transformed and again examined for normality using the Shapiro-Wilk Test. Results showed that the log-transformed concentration data did not depart significantly from normality (p = 0.10 for both diazinon and chlorpyrifos), indicating that both the diazinon and chlorpyrifos concentration data could be assumed to be lognormally distributed. Thus, all analysis of residential diazinon and chlorpyrifos data assumed a lognormal distribution.

Since the final mass load estimates involve pooling of the residential data, it was necessary to assess whether variances were homogenous among neighborhoods. The log-transformed data of R01-R05 were tested for homogeneity of variance using both the Bartlett's Test and the Levene's Test. The Bartlett's Test gave the p-values for diazinon and chlorpyrifos of 0.33 and 0.12, respectively. The Levene's Test gave the p-values for diazinon and chlorpyrifos of 0.81 and 0.45, respectively. Results for both tests were not significant indicating that the variances from the five neighborhoods were not significantly different.

As with the influent, the best estimate of the true mean (a) of the untransformed, lognormally distributed residential concentration data was the UMVU estimator. The geometric mean was not chosen since it estimates the median, rather than the mean of a lognormal distribution. For highly skewed distributions, the use of the geometric mean can result in the underestimation of the true mean (a). Since some of the residential data sets were highly skewed, the underestimation of the true mean (a) can be pronounced. Based on the need to control for negative bias, the UMVU estimates of daily diazinon concentrations were calculated for R01-R05 (Table 5).

Table 5: UMVU Estimates of True Daily Mean (a) Residential Diazinon and Chlorpyrifos Concentrations

Area #	Location	Residential Area	a-Estimate of Residential Diazinon Concentration (ng/L)	a-Estimate of Residential Chlorpyrifos Concentration (ng/L)
R01	2nd Ave. South	Martinez/Pacheco	740	550
R02	Turtle Rock Ln.	Concord	420	110
R03	Acalanes Rd.	Lafayette	120	80
R04	Miner Rd.	Orinda	110	110
R05	Greenbrook Ct.	Danville	340	180

Diazinon concentrations in residential samples ranged from none-detected to 4,300 ng/L. R01 had the highest estimate of the true daily mean (a) diazinon concentration among the sampled neighborhoods at 740 ng/L. This was strongly influenced by the occurrence of the 4,300 ng/L on the second day of residential sampling. R02 and R05 had the second and third highest a-estimates, respectively. The a-estimates for R02 and R05 differed by 80 ng/L; however, the R02 1-day peak of 2,200 ng/L was almost three times larger than the R05 peak of 770 ng/L. The R03 a-estimate of 120 ng/L was slightly larger than the R04 a-estimate of 110 ng/L. Diazinon levels at R04, however, were more frequently above reporting limit than at R03.

Chlorpyrifos concentrations in residential samples ranged from none-detected to 1,200 ng/L. The four highest daily chlorpyrifos concentrations (1,200, 1,200, 490, and 410 ng/L) also occurred at R01. Subsequently, R01 had the highest estimate of the true daily mean (a) chlorpyrifos concentration at 550 ng/L. The second highest a-estimate of 180 ng/L at R02 was 370 ng/L less than the R01 estimate. R02 and R04 have the identical a-estimates of 110 ng/L. Chlorpyrifos concentrations at R02, however, were more frequently above the reporting limit than at R04. R03 had the lowest a-estimate among the neighborhoods sampled at 80 ng/L.

Notable differences between daily concentrations at the same sites suggested that the diazinon and chlorpyrifos in the sewer may have traveled in pulses (Table F2 in Appendix F). Thus, it is possible that there was variability in concentrations of the 24 subsamples that were composited to achieve the 4,300 ng/L diazinon sample from R01. For example, if the loading occurred over six hours, the concentration in each of the six bottles would have been about 25,800 ng/L. However, if the pulse was captured in just one bottle, the resulting concentration in that bottle would have been about 103,000 ng/L or 103 parts per billion. A concentration of this magnitude would have surpassed the concentrations found at any of the commercial sites monitored in this study. Unfortunately, the 24 subsamples from July 11, 1996, were discarded after compositing.

The diazinon concentration of 4,300 ng/L and flow rate of 490,000 gal/day amounted to about 8.0 g of diazinon active ingredient. Considering the more common forms of diazinon for home use, this amount would be equivalent to about 2 tablespoons of diazinon aqueous concentrate (25% active

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ingredient) or about 2½ gallons of a ready-to-use diazinon liquid solution (0.75% active ingredient). For comparison, if the lowest diazinon concentration detected at RO 1 (110 ng/L) was back-calculated, the resulting mass of diazinon active ingredient from the neighborhood would be about 0.20 g. This is equivalent to about 1/7 of a teaspoon of diazinon aqueous concentrate (25% active ingredient) or about 8 fl. oz. of a ready-to-use diazinon liquid solution (0.75% active ingredient).

Diazinon loading on the first and last day of the residential sampling week was about 40 times smaller than the loading on July 11, 1996. Moreover, a comparison of ROl and CCCSD influent diazinon mass loads on that day shows that ROl (which comprises approximately 1% to the influent flow), accounted for about 18% of the influent mass load. However, it is difficult to determine the exact nature of diazinon loading that occurred on that day without additional source information. For example, 8.0 g of diazinon active ingredient could have resulted from a major disposal (i.e. pouring unused portions of diazinon down the drain), a myriad of smaller introductions (i.e. hand washing or laundering of contaminated clothes), or any combinations in between. Similar possible loading scenarios should also be recognized for chlorpyrifos. The highest residential chlorpyrifos daily mass input of 2.2 g occurred on July 12, 1996 at R01. This mass represented about 10% of the influent chlorpyrifos mass load on that day. With over 2,000 residences in RO 1, small contributions of diazinon and chlorpyrifos from each home to the sewer can become collectively significant.

During residential sampling week, ROl-R05 contributed as little as 1% and as much as 22% of the CCCSD daily influent diazinon load. For chlorpyrifos, RO 1 -R05 contributed as little as 2% and as much as 10% of the CCCSD daily influent load. The majority of the mass loads originating from the five residential areas can be attributed to ROl as shown in Figures 8 & 9.

Figure 8: Sources of ROI-R05 Diazinon Mass (7/9-7/15/96)
R05 11%
R04 3%
R03 4%
R02 24%
ROI 58%

Figure 9: Sources of ROI-R05 Chlorpyrifos Mass (7/9-7/15/96)
R05 11%
R04 7%
R03 3%
R02 11%
R01 68%
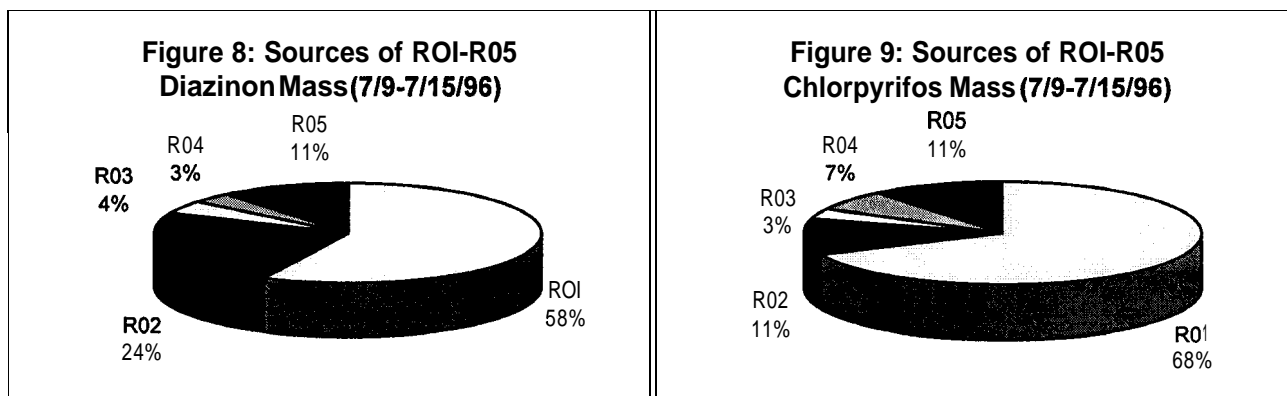
Figures 10 & 11 present the same residential diazinon and chlorpyrifos concentrations as shown in Figures 6 & 7; however, the data were arranged in two dimensions to facilitate day-of-the-week comparisons. The highest diazinon concentrations for each neighborhood all occurred from Wednesday to Friday. For chlorpyrifos, the highest concentrations occurred on Monday, Wednesday and Friday. None of the peaks in each neighborhood occurred on the Saturday or Sunday of the residential sampling period.

Figure 10: Daily Residential Diazinon Concentrations (7/9-7/15/96)

Figure 11: Daily Residential Chlorpyrifos Concentrations (7/9-7/15/96)
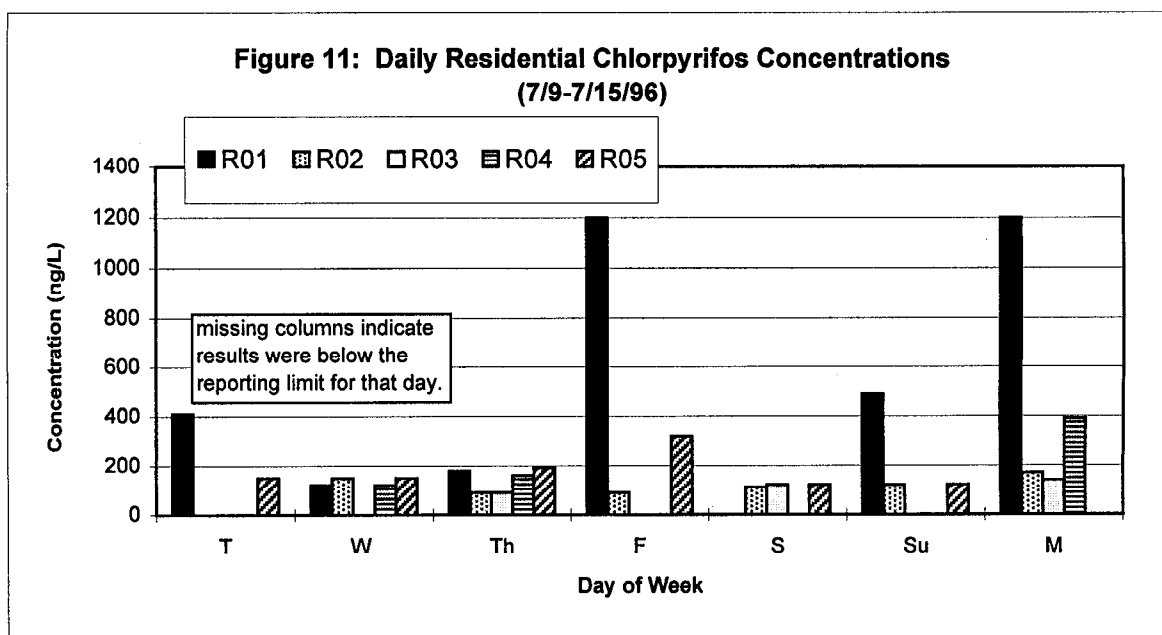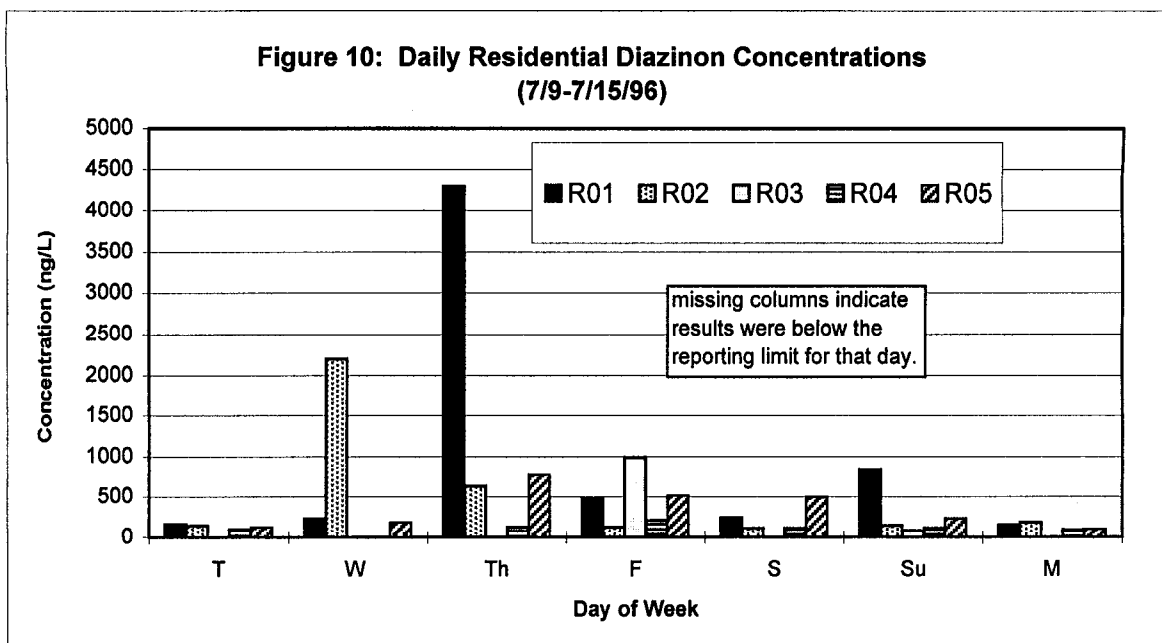
Personal and professional use of organophosphates at residences are temporally variable. Thus, assuming these are major sources of diazinon and chlorpyrifos in residential areas, diurnal residential input patterns can also be variable. For example, gardening is not limited to weekends, but is also a popular weekday activity. Pet care activities (e.g. flea spraying, pet washing) occur throughout the week and are not limited to daylight hours. Professional applications of organophosphates by pest control operators and landscape maintenance services take place throughout the week. Surveys on diazinon and chlorpyrifos residential uses are required to characterize temporal variations in residential sources. If these uses can be linked to transport mechanisms into the sewers, then perhaps the observed residential concentration patterns of diazinon and chlorpyrifos can be better understood.

Three-hour composite samples were taken at R02 from August 13-14 to capture the loading profile during a 24-hour period (Figure 12 on following page). Detectable concentrations of diazinon occurred between 10 a.m. and 10 p.m. with the highest concentration of 360 ng/L occurring from 10 a.m. to 1 p.m. During this profile, inputs of diazinon at R02 appeared to be occurring from early morning through early evening hours which reflects diurnal use patterns. Unlike diazinon, chlorpyrifos was detected in all eight samples from the 24-hour period. The highest concentration (500 ng/L) occurred from 4 p.m. to 7 p.m. Notable concentrations of 410 ng/L and 310 ng/L persisted from 7 p.m. until 1 a.m. This continuous profile may have simply resulted from continuous input. Alternatively, it may be related to the more pronounced chemical retention of chlorpyrifos in sewer pipes relative to diazinon.

CCCSD has suspected that diazinon and chlorpyrifos may be absorbing into the layers of organic material that line the inside of many sewer conduits (CCCSD, 1995). Once absorbed, the active ingredients can be released back into the waste stream. The higher affinity of chlorpyrifos (Kow = 50,100) (DPR, 1995) for organic substances compared to diazinon (Kow = 1980) may result in an increase in residence time for chlorpyrifos. The limited monitoring results from R02 appear to support this hypothesis; however, bench scale testing and/or tracer dye studies should be performed to provide a more definitive answer.

Figure 12: Diazinon and Chlorpyrifos Concentrations from 8/13-8/14/96 at R02
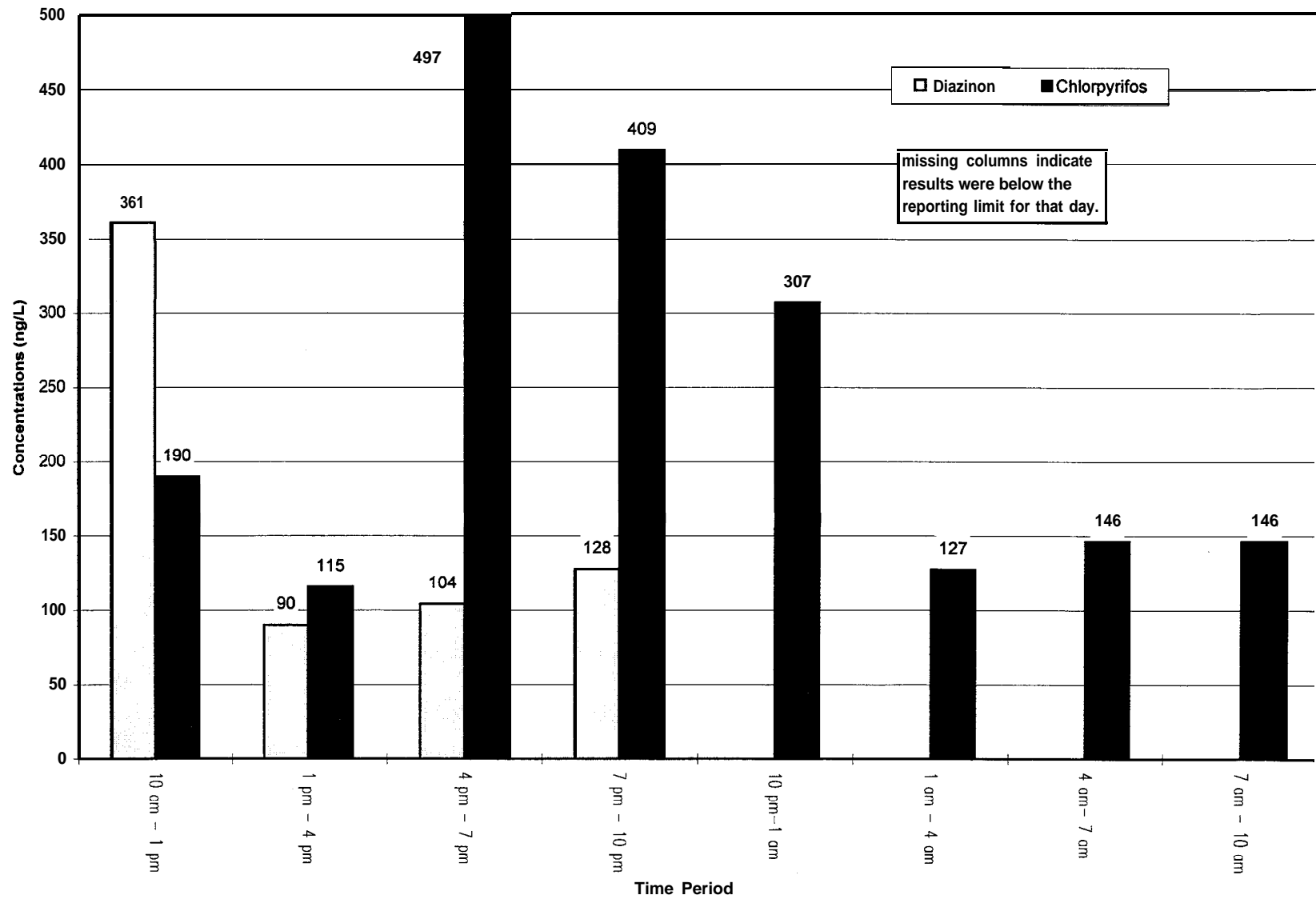
Commercial sampling took place from July 18 through September 8 at 12 different sites within the CCCSD collection system. These sites included five pet groomers, five pest control operators, and two kennels. Samples were taken based on the days of the week in which heaviest input was expected. The total number of days sampled at each site varied from 1-4 days. None of the field and equipment blanks associated with the commercial sites contained detectable amounts of either active ingredients. Table F3 in Appendix F contains the raw and recovery-adjusted commercial sampling results.

The highest and lowest concentrations observed in the commercial sampling span several orders of magnitude. The small number of samples from each site limits the use of statistical summary values and tests. Ten of the 12 sites have three or fewer observations. Based on the observations from the influent and residential sources, the distribution of commercial sources could be assumed to conform to a lognormal distribution. However, the use of UMVU estimator of a for a lognormally distributed population cannot be calculated for this data set because the small sample sizes at most of the commercial sites do not permit adequate characterization of the distribution. Therefore, the discussion of commercial results was based simply on the individual daily concentration data.

Both diazinon and chlorpyrifos were detected at 7 of the 12 sites monitored (Figures 13 & 14 on the following pages). At two sites, only diazinon was detected. At two other sites, only chlorpyrifos was detected. The only site at which neither active ingredient was detected was C09, a pet groomer. The mixed nature of the results suggests that diazinon and chlorpyrifos input levels can vary greatly among commercial sites.

The highest diazinon concentration (20,000 ng/L) was found at a kennel (C11). This site also had the second to fourth highest diazinon concentrations of 14,000, 13,000 and 13,000 ng/L. Other notable detections of diazinon were made at pet groomer C10 (1,000 ng/L), PCO C14 (760 ng/L), and PCO C16 (1,100 ng/L). Diazinon levels were above the reporting limit on 17 of the 32 days sampled.

Figure 13: Daily Diazinon Concentrations at Sampled Commercial Sites

C06-C10 = pet groomers
C11-C12 = kennels
C13-C17 = pest control operators

missing columns indicate that results were below the reporting limit for that sampling day.

29
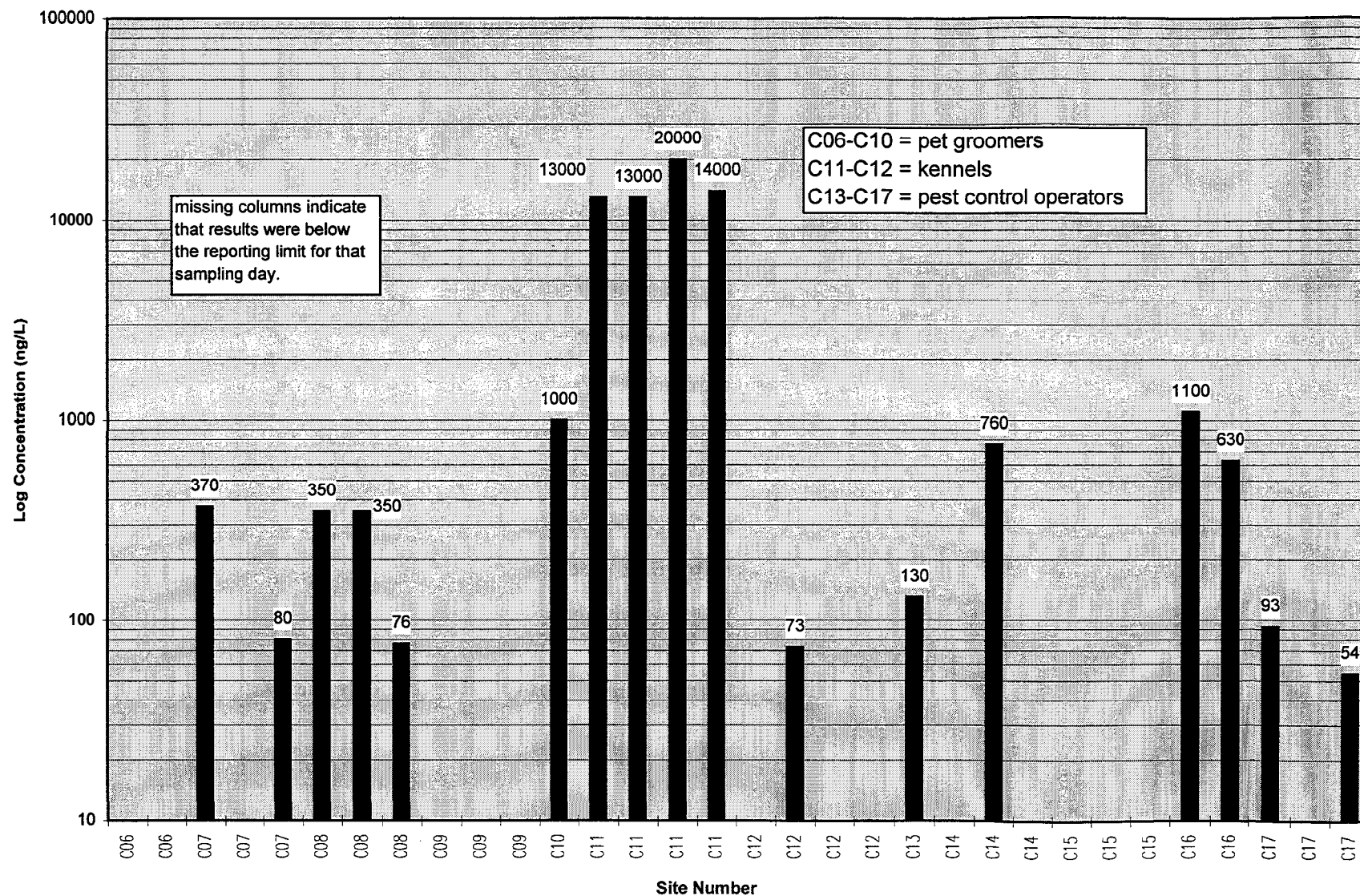
Figure 14: Daily Chlorpyrifos Concentrations at Sampled Commercial Sites
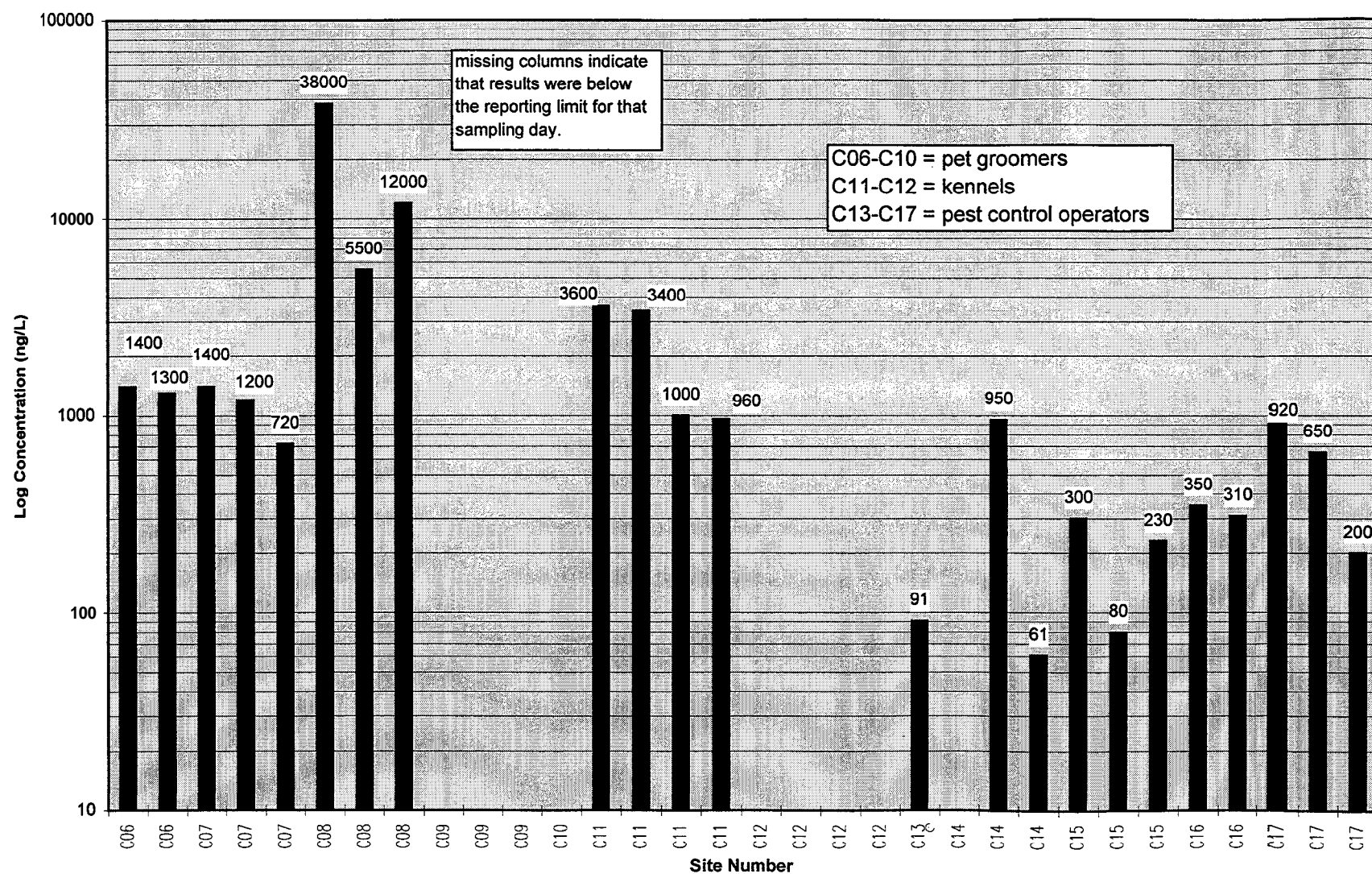
The highest chlorpyrifos concentration (38,000 ng/L) was found at a pet groomer (C08). This site also had the second and third highest chlorpyrifos concentrations at 12,000 and 5,500 ng/L. Other detections at or above 1,000 ng/L were found at pet groomer C06 (1,400 and 1,300 ng/L), pet groomer C07 (1,400 and 1,200 ng/L), and kennel C11 (3,600, 3,400, and 1,000 ng/L). Chlorpyrifos levels were above the reporting limit on 23 of the 32 days sampled.

Even sites within the same commercial groups (e.g. kennels versus kennels) were quite variable. For example, the lowest daily diazinon concentration found at C11 (a kennel) was 13,000 ng/L. At C12 (also a kennel) the highest daily diazinon concentration measured over the 4-day sampling period was 73 ng/L. Likewise, C08 and C09 (both pet groomers) also differed in chlorpyrifos concentrations. The lowest daily concentration at C08 was 5,500 ng/L, while chlorpyrifos was not detected on any of the days sampled at C09.

Diazinon concentrations of 20,000 ng/L at C11 surpassed the 4,300 ng/L concentration that was the maximum residential level. Commercial chlorpyrifos levels of 38,000 ng/L at C08 also surpassed the 1,200 ng/L at R01. However, the flow at C11 and at C08 were relatively low compared to the flow of R01. The diazinon level of 20,000 ng/L at C11, coupled with the flow that day of 3,274 liters, only translated to approximately 0.065 g of active ingredient. At C08, the 38,000 ng/L of chlorpyrifos and the flow of 10,905 liters was equivalent to about 0.41 g. The highest diazinon and chlorpyrifos daily masses at R01 were about 8.0 g and 2.2 g, respectively. However, note that residential sampling sites such as R01 actually represent a sum of many individual residences. If the average mass loads of each home in the R01 area were calculated, the daily mass loads from C11 and C08 would appear more significant on a per-structure basis. Recall that the 8.0 g of diazinon active ingredient at R01 accounted for 2,079 residences. Thus, a per-residence mass would be about 0.0038 g. This amount is about 17 times smaller than the peak diazinon input at C11.

The chlorpyrifos level of 38,000 ng/L at C08 was equivalent to 0.41 g of active ingredient. Since C08 was a pet grooming business, this amount of pure active ingredient was equivalent to about 0.62 fl. oz. or about 4 teaspoons of a flea and tick dip with 2.0% chlorpyrifos. An informal site survey done by CCCSD in September 1993 found that a particular dog grooming business was using a

chlorpyrifos flea dip about once a week (Pomroy, March 1994). The survey documented that 2 fl. oz. of a 2.0% chlorpyrifos flea dip was used. Considering that the active ingredient in the dip was intended to stay on the animal, it is plausible that the loading estimate of 0.62 fl. oz. (about 31% of the chlorpyrifos applied) would have made it off the animal and into the drain. Chlorpyrifos may also be released from a pet groomer via shampooing and regular rinsing of animals which had previously been exposed. Therefore, even the highest commercial chlorpyrifos concentration found in this study can result from common practices.

When the site specific flow measurements were used with the daily concentration levels to calculate the actual input mass of chlorpyrifos, it is apparent that certain commercial sources dominate. Approximately 72% of the total mass produced from these commercial sources over the 32 days of sampling can be attributed to just 3-days of sampling at pet groomer C08. Similarly, 3-days of sampling at kennel C11 produced 41% of the study's total commercial diazinon mass. In this limited study of commercial businesses, a handful of sources provided the majority of commercial inputs.

Publicly-Owned Treatment Works

The influents of Union Sanitary District (USD) and the Palo Alto Regional Water Quality Control Plant (RWQCP) were sampled concurrently with the CCCSD influent from August 5-11, 1996. The CCCSD influent concentrations used in this comparison are a subset of the data previously discussed in the CCCSD influent section. The synchronization scheme among the POTWs minimized temporal variability. Table F4 in Appendix F contains the POTW sampling results.

Sampling methods used among the three locations were similar, with the exception that the recycle flow at RWQCP also had to be monitored. USD's results from August 5 were not plotted or used in subsequent calculations because the recycle flow on that particular day showed 1100 ng/L of diazinon and 230 ng/L chlorpyrifos. USD staff reported that the elevated diazinon and chlorpyrifos concentrations in the recycling flow was likely due to the partial system cleaning conducted just prior to the first day of POTW sampling. POTW monitoring results are plotted in Figures 15 & 16.

Figure 15: Daily Influent Diazinon Concentrations at CCCSD, USD, and RWQCP (8/5-8/11/96)

8/5/96 data omitted due to possible contamination from recycle flow.
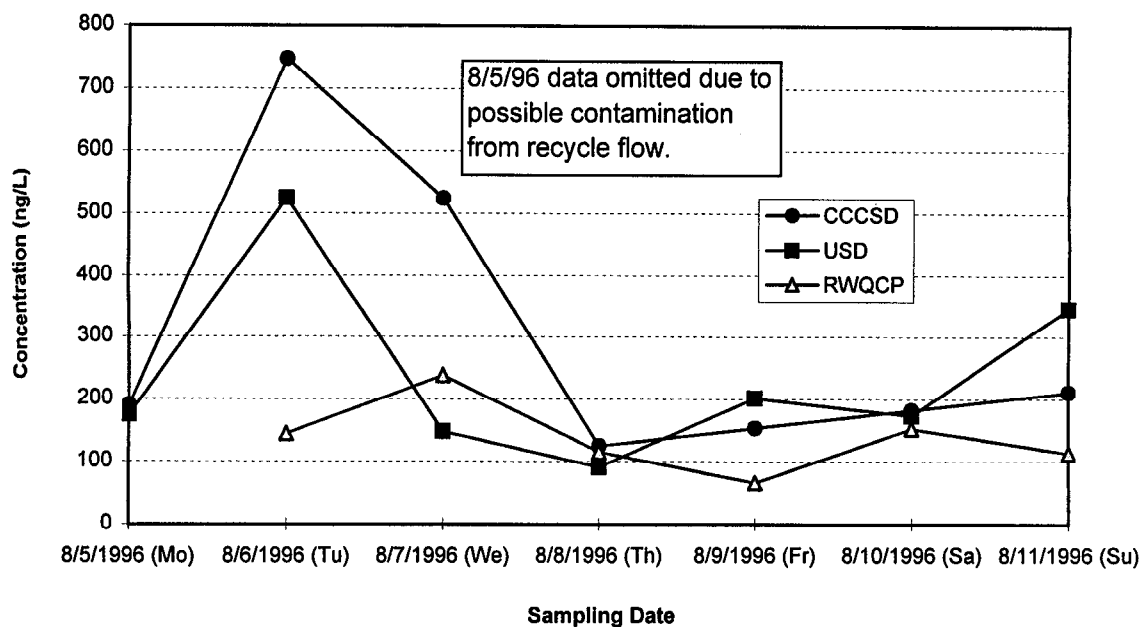
Figure 16: Daily Influent Chlorpyrifos Concentrations at CCCSD, USD, and RWQCP (8/5-8/11/96)

8/9/96 data not plotted because below reporting limit

8/5/96 data omitted due to possible contamination from recycle flow.

33
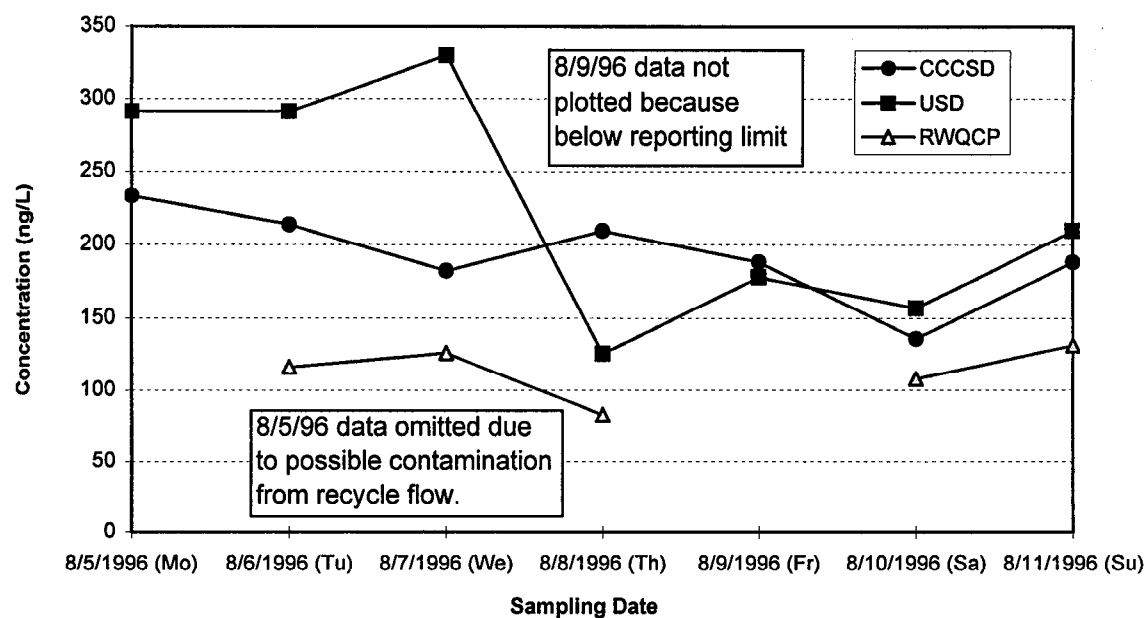
During the sampling week, the highest and lowest diazinon concentrations at each POTW occurred on weekdays. Interestingly, CCCSD and USD both reached their peak concentration on Tuesday, and their lowest concentration on Thursday. For chlorpyrifos, two of the three POTWs had their peak concentrations on a weekend day. The lowest concentrations for all three POTWs occurred on weekdays.

The influent diazinon and chlorpyrifos concentrations of USD and RWQCP were assumed to be lognormal. This is based on the finding that CCCSD's influent diazinon and chlorpyrifos concentrations were also lognormally distributed. The UMVU estimators of daily influent concentrations of diazinon and chlorpyrifos were calculated for each POTW using data from August 5-11, 1996 (Table 6).

Table 6: UMVU Estimates of True Daily Mean (a) Influent Diazinon and Chlorpyrifos Concentrations of CCCSD, USD, and RWQCP

POTWs	a-Estimate of Influent Diazinon Concentration (ng/L)	a-Estimate of Influent Chlorpyrifos Concentration (ng/L)
Central Contra Costa Sanitary District (CCCSD)	300	190
Union Sanitary District (USD)	230	230
Palo Alto Regional Water Quality Control Plant (RWQCP)	150	110

Influent diazinon concentrations during this period for CCCSD, USD, and RWQCP ranged from 130 to 750 ng/L, 91 to 530 ng/L, and 66 to 240 ng/L, respectively. The corresponding estimates of the true daily mean (a) influent diazinon concentrations for the three POTWs were 300, 230, and 150 ng/L, in the same order. A One-Way ANOVA Test on the log-transformed data and a Kruskal-Wallis Test (non-parametric ANOVA) on the non-transformed data were performed. Both tests compare the ratio of the median values (not the means) of each POTW to determine if differences exist. Results of both tests showed no significant difference between the median diazinon concentrations of the POTWs ($p > 0.15$).

Influent chlorpyrifos concentrations in this same period for CCCSD, USD, and RWQCP ranged from 140 to 230 ng/L, 130 to 330 ng/L, and none-detected to 150 ng/L, respectively. The corresponding estimates of the true daily mean (a) influent chlorpyrifos concentrations for the three POTWs were 190, 230, and 110 ng/L, in the same order. The same statistical tests used for the diazinon data were used to test median chlorpyrifos concentrations. Both tests determined that CCCSD and USD were not significantly different from one another. However, the test showed that RWQCP's median chlorpyrifos concentration was significantly lower than the other POTWs. The p-values for both tests were <0.001. RWQCP's median influent chlorpyrifos concentration was about half of the influent median concentrations of CCCSD and USD.

It is not clear why RWQCP's influent showed a significantly lower median concentration of chlorpyrifos than CCCSD and USD. The disposal practices, physical characteristic of the collection system, source compositions, flow, and many other factors are unique to a POTW, producing the observed influent concentrations. A study of the factors that lead to the lower median chlorpyrifos concentrations of RWQCP's influent may perhaps lead to useful discoveries that can help reduce concentrations in other sewage collection systems. On the other hand, such an observation may simply be a result of lower chlorpyrifos usage in the RWQCP's service area.

Mass Balance Calculations

CCCSD Influent Mass Load Estimates - Flow data from the influent flowmeter were available for each day of the sampling. However, the SNAP modeling program data were used instead because the same program was used to generate residential flow data. For CCCSD's influent, the SNAP flow value was 38,400,000 gal/day. As a comparison, the daily average from the influent flowmeter between July 9-15 was 38,847,429 gallons. There was only a one percent difference of model flow to actual flow.

Concentration values were obtained from 24-hr. flow-composited samples. Recall that the July 9-15, 1996 subset of the influent data was used specifically because residential sampling also occurred during this week. The daily influent concentrations between July 9-15 are considered to be

lognormal because they are a subset of the lognormally distributed total influent data. Thus, the best estimate of the daily influent mean during this period was also the UMVU estimator.

The SAS® program estimated the mean daily influent concentrations for July 9-15, 1996 to be 330 ng/L for diazinon and 170 ng/L for chlorpyrifos. These concentrations and the fixed flow of 38,400,000 gal/day were multiplied giving the mean daily mass loads at the influent of 48 g for diazinon and 25 g for chlorpyrifos (Table 7).

Table 7: Estimates of Daily CCCSD Influent Diazinon and Chlorpyrifos Mass Load (with Land 95% Confidence Intervals)

UMVU Estimate of True Daily Mean (α) Influent Concentrations (ng/L) between 7/9/97-7/15/97		SNAP-Generated Average Daily Influent Flow		Estimate of Daily Mass Loads (g) between 7/9/97-7/15/97	
diazinon	*chlorpyrifos*	*gallons*	*liters*	*diazinon*	*chlorpyrifos*
(270-**330**-420)	(140-**170**-200)	38,400,000	145,359,360	(39-**48**-61)	(20-**25**-29)

In addition to the true daily mean estimates, confidence intervals for diazinon and chlorpyrifos were also calculated. There are several methods available for computing the interval estimates of the true mean (α) of a lognormal distribution; however, only the method developed by Land (1971) gives exact intervals (Ung and Vegiard, 1988). The SAS® control file executes a Fortran program developed by Land et al. (1987) to calculate exact confidence intervals for the true mean (α) of both diazinon and chlorpyrifos. The 95% Land exact confidence intervals are also presented in Table 7. The asymmetric confidence intervals are the result of the skewness of the lognormally distributed influent population.

Residential Mass Load Estimates - Before residential mass loads could be estimated, the overall mean diazinon and chlorpyrifos concentrations had to be calculated from the five neighborhood α-estimates from Table 5. The arithmetic mean or median was not used to calculated the overall R01-R05 mean. This was because R01-R05 were not specifically selected based on how they accurately reflect (in terms of flows and concentrations) the profile of all neighborhoods in the CCCSD service

area. Instead, the UMVU estimator was used to estimate the overall true daily mean for all residential neighborhoods using the five neighborhood a-estimates to characterize the distribution of mean concentrations. The SAS® program estimated the overall residential a to be 350 ng/L for diazinon and 200 ng/L for chlorpyrifos.

The appropriate flow value then had to be selected. Although actual flow readings from R01-R05 were taken, the suspicion that some of the flow data were erroneous led to the consideration of other flow determination alternatives. The SNAP-generated flow data was selected since they were verified by CCCSD to be accurate. The availability of the SNAP-generated flow for use on the influent side of the mass balance equation maintained consistency and further supported its use. Thus, the SNAP-generated flow of 31,600,000 gal/day was determined to be most representative of the daily total residential flow. The total daily residential mass load estimates for diazinon and chlorpyrifos are shown in Table 8.

Table 8: Estimates of Daily Residential Diazinon and Chlorpyrifos Mass Loads (with Land 95% Confidence Intervals)

UMVU Estimate of Overall True Daily Mean (a) Residential Concentrations (ng/L) between 7/9/96-7/15/96		Total SNAP-Generated Total Daily Residential Flow		Estimate of the Total Daily Residential Mass Loads (g)	
diazinon	*chlorpyrifos*	*gallons*	*liters*	*diazinon*	*chlorpyrifos*
(160-**350**-3,000)	(100-**200**-1,200)	31,600,000	119,618,640	(37-**42**-360)	(12-**24**-140)

The Land 95% confidence intervals for the true mean (a) were also calculated for residential estimates. The width of the intervals (particularly of diazinon) is quite large due to the large variation in the neighborhood a-estimates.

The initial comparison of residential mass to influent mass indicated that about 87% of the diazinon and 96% of the chlorpyrifos were attributable to residential areas. For comparison, if mass contributions from residential sources were calculated based on the unadjusted raw data, these

sources would account for about 56% of total influent diazinon and 63% of total influent chlorpyrifos. Although the raw data are considered to be conservative due to the lower recoveries, they still show that more than half of the daily influent mass loads for both active ingredients are residential in origin.

When calculating percent contributions, natural loss of both diazinon and chlorpyrifos within the collection system should be considered. An analyte stability study conducted by AQUA-Science shows that approximately 15% of the diazinon and 14% of the chlorpyrifos initial concentrations were lost within the first 24 hours of introduction into the sewage matrix (AQUA-Science, 1995). Thus, it is likely that the same diazinon or chlorpyrifos molecule measured upstream may not be accounted for downstream at the influent due to loss via various abiotic and biotic (microbial) processes. Hydrolysis, volatilization, particle sorption, oxidation, and hydroxylation are some pathways that are responsible for the loss of organophosphates from aqueous solutions (Chambers and Levi, 1992). Factors such as pH, temperature, and microbial activities can also influence the degradation kinetics of organophophates (Lartiges and Garrigues, 1995). Therefore, the true residential contribution percentages of diazinon and chlorpyrifos are likely to be somewhat lower than the initial estimates. Moreover, AQUA-Science's data were generated for the purpose of determining loss under storage conditions. Thus, samples were stored at 4^E C. A degradation study that mimics conditions within the sewers (temperature, pH, agitation, etc.) may provide data which can be used to more accurately estimate the loss diazinon and chlorpyrifos during transit.

Commercial Mass Load Estimates - The UMVU estimator was not used to estimate commercial mass loads since it was deemed to be inappropriate for use with the very small sample sizes (Blackwood, 1992). Instead, the estimated total daily commercial mass loads were based on the arithmetic mean daily mass loads from each site sampled. The arithmetic mean was used in this case because existing datasets (1-4 values from each site) were limited. The arithmetic mean is the best alternative to obtain an unbiased estimate of the true mean (a) when sample sizes are small (Blackwood, 1992).

Site-specific daily masses were possible to calculate since synchronous flow measurements were

available. For practicality, 25 ng/L (one-half the RL) was substituted in calculations for non-detects. The total daily mass input from each class (pet groomer, kennels, and PCOs) was then extrapolated. CCCSD estimated 29 pet groomers, 24 kennels, and 148 PCOs as potential sources in the service area. This projection is based on the assumption that the concentration and flow profiles of sampled sites were fairly representative of the overall commercial cross-section.

The total daily mass of the three business types were summed to represent the total commercial mass (Table 9). The use of the term "commercial" is subjective and refers to businesses classified as a pet groomer, kennel, or PCO. Other businesses that are sources of diazinon and chlorpyrifos certainly exist although their mass loads may not be included as part of the "commercial" estimate.

Table 9: Estimates of Daily Commercial Diazinon and Chlorpyrifos Mass Loads

Business Type	Sum of the Daily Mean Mass of Sampled Sites in Each Class (g)		Number of Commercial Sites		Estimate of the Total Daily Commercial Mass Loads (g)	
	diazinon	*chlorpyrifos*	*sampled*	*service area total*	*diazinon*	*chlorpyrifos*
Pet Groomers	0.0417	0.2542	5	29	0.2419	1.475
Kennels	0.0357	0.0073	2	24	0.4288	0.0877
PCOs	0.0530	0.0263	5	148	1.568	0.7779
Total					**2.2**	**2.3**

Due to the small commercial sample set, it was not possible to calculate the confidence intervals for the commercial estimates. Although this estimate is short on statistical strength as compared to the influent and residential estimates, consider the following scenario: For the 201 commercial sources to produce the equivalent amount of diazinon and chlorpyrifos as all the residential sources (values in Table 8), they would have to contribute a daily diazinon concentration of about 3,200 ng/L and daily chlorpyrifos concentration of about 1,600 ng/L, assuming that they release an average flow of about 29,500 L/day. This flow term is the average flow of the 12 sites sampled. Existing commercial results showed that only one of the twelve sites sampled exhibited the capability of surpassing such a level for diazinon. Likewise, only two of the twelve sites sampled showed the

same capability in exceeding the estimated chlorpyrifos level. None of these sites were PCOs, who comprised 74% of the commercially-classified sources in the CCCSD service area. Since each sample site occasionally represents flows from other commercial businesses, the 29,500 L/day average used in the previous calculation may be inflated. The concentrations of diazinon and chlorpyrifos required to produce an equivalent mass to the sum of all residential sources may even be higher. Based on this analysis and the previous residential mass load estimates, mass contribution from commercial sources appeared minor compared to residential sources.

The total daily commercial diazinon and chlorpyrifos mass load estimates (2.2 g and 2.3 g, respectively) were quite close (Table 9). Initial comparison of commercial to influent mass loads suggested that about 5% of diazinon mass and 9% of chlorpyrifos mass were attributable to pet groomers, kennels, and PCOs in the service area. If loss during transit was considered for commercial loading, the percent contribution figures would be less than the percentages calculated. The calculation of commercial percentage contribution assumed that the daily commercial loading patterns were identical during the period in which the influent loads were quantified.

Unaccounted Mass Loads - Residential and commercial flows made up 88% of the CCCSD influent flow. Their estimated masses combined for approximately 92% of the diazinon and 105% of the chlorpyrifos of the service area (Figure 17). Consequently, only about 4 g of diazinon (8% of influent mass) still remain unaccounted for. The sum of residential and commercial chlorpyrifos inputs is actually greater than the amount received at the influent. The degradation of chlorpyrifos and diazinon during transit will likely reduce the projected percent contributions; however, there may be other reasons as to why the combined percent contributions were so high.

Figure 17: Estimated Residential and Commercial Contributions to CCCSD's Influent Diazinon and Chlorpyrifos Mass Loads (not adjusted for loss)
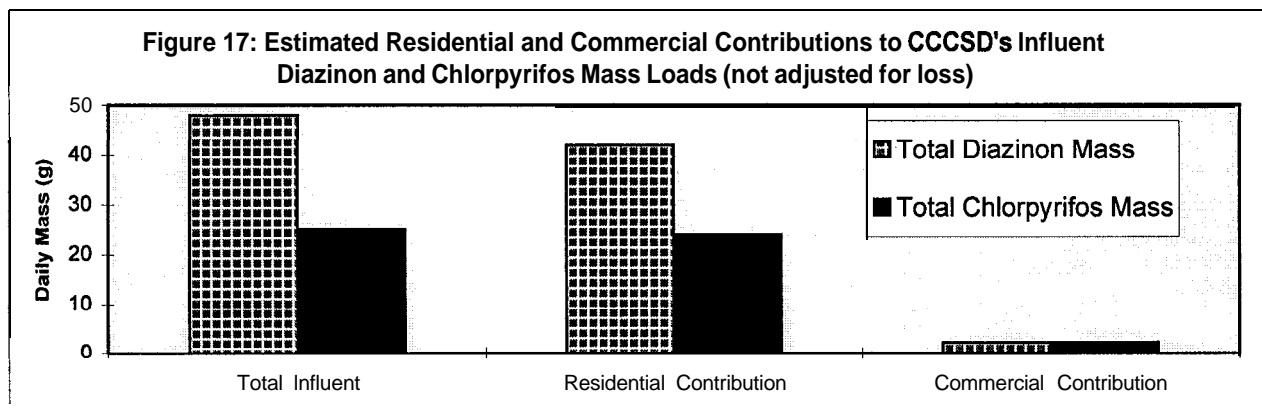
In mass balance equations, all calculated mean concentrations and flow values are best-substitutes for the true mean values. The limited numbers of samples collected did not necessarily provide an exact representation of all the sources in the service area. For example, if the 4,300 ppt concentration of diazinon at R01 was a rare occurrence or error, it would have raised the estimate of the overall true daily mean (a) residential concentration to a value that is actually higher than the true value. The assumption and subsequent use of this estimate to represent the typical CCCSD service area neighborhood would then result in an overestimation. Similar errors in projection can also affect the influent mass estimates. For example, a difference of 5 ng/L in active ingredient will change the influent mass estimates by 1.5% for diazinon and 3.0% for chlorpyrifos. Small changes in concentration values translate into large changes in mass values when the flow term used in the mass loading calculation is large. The assumption must be made that the sites are representative of their source categories in order to argue that the mass contribution estimates are reasonable. The results from this study should be used only to rank contributions from the source categories, and as background to plan further monitoring studies.

Although projections show that residential and commercial (PCOs, pet groomers, and kennels) sources represent the majority of inputs into the collection system, unaccounted diazinon and chlorpyrifos may have also originated from unmonitored sources. For example, little or no monitoring data is available on potential commercial sources such as restaurants and nurseries. CCCSD also receives the effluents of many industrial facilities. A grab sample taken by CCCSD at a local waste management facility showed high concentrations (in the parts per billion or µg/L range) of diazinon in the facility's wastewater holding tank. Industrial effluents combine for a total flow of about 1.7 million gallons per day which is destined for the CCCSD treatment plant. There are also land uses which were not sampled (e.g. golf courses, parks, and resort areas) that are likely to use higher amounts of the two active ingredients.

POTW Mass Load Estimates - The mass load estimates of CCCSD, USD, and RWQCP were calculated using the UMVU estimators generated from samples collected between 8/5/96 and 8/11/96. The average influent flow rates of each POTW were derived from flowmeter readings

during the same time period. Thus, CCCSD's daily mass load estimates from this period are not identical to those shown in Table 10 which were derived from data between 7/9/96 and 7/15/96. Table 10 presents the mass load estimates of the three POTWs as well as the Land 95% confidence intervals. Note that these intervals are not ranges of the concentrations measured.

Table 10: Comparison of POTW Influent Mass Load Estimates (with Land 95% Confidence Intervals)

POTW	UMVU Estimate of the True Daily Mean (a) Influent Concentrations (ng/L) between 8/5/96-8/11/96		Average Measured Daily Flow	Estimate of the Daily Mass Loads (g) between 8/5/96-8/11/96	
	diazinon	*chlorpyrifos*	*liters*	*diazinon*	*chlorpyrifos*
CCCSD	(180-**300**-780)	(170-**190**-230)	147,053,058	27-**44**-115	25-**28**-34
USD	(150-**230**-500)	(170-**230**-340)	105,234,120	16-**24**-53	18-**24**-36
RWQCP	(100-**150**-240)	(80-**110**-170)	92,623,329	9.3-**14**-22	7.4-**10**-16

The estimated daily mass loads at CCCSD, USD and RWQCP were 44, 24, and 14 g, respectively. The average daily mass of 44 g of diazinon entering the CCCSD plant each day was more than the USD and RWQCP average daily inputs combined. At this rate, CCCSD would receive a kilogram of diazinon active ingredient in about 23 days. A kilogram of the active ingredient is equivalent to roughly 250 tablespoons of a diazinon aqueous concentrate (25% active ingredient). This amount is significant when considering a recent study on the removal rate for diazinon suggested that the current CCCSD treatment process reduces influent diazinon by only 32 percent (Lai, November 1996).

The same study found that USD's influent diazinon concentrations and mass loads are also fairly high. USD's diazinon removal rate was determined to be about 24%. When this removal rate was applied to the estimated USD daily influent mass load, the resulting effluent mass was less than that of CCCSD. The same study revealed that the treatment process at RWQCP produced a diazinon removal rate of 82%. The following factors may have contributed to RWQCP's higher removal rate of diazinon over the other two POTWs: 1) the use of a fixed film/activated sludge system, 2) longer

sludge age, 3) longer chlorine contact time, and 4) the use of a dual media filtration system (tertiary treatment)(Lai, November 1996).

The daily average chlorpyrifos mass loads at CCCSD, USD and RWQCP were approximately 28, 24, and 10 g, respectively. At 28 grams per day, it would take about 36 days for a kilogram of chlorpyrifos to enter the CCCSD influent. RWQCP removal rates for chlorpyrifos (71 percent) was the highest of the three POTWs owing mostly to the same factors cited for diazinon. Chlorpyrifos removal rates for both CCCSD (53%) and USD (49%) mean that about half of the influent chlorpyrifos remained in the effluent.

CONCLUSIONS

Mass balance estimates indicate that residential sewage contributes the majority of the diazinon and chlorpyrifos to CCCSD's influent. Relatively high concentrations were found at commercial sources; however, low commercial flows can be expected to produce smaller contributions of mass. Unaccounted sources of chlorpyrifos and diazinon may also exist. Uninvestigated sources include restaurants, nurseries, industrial facilities, and isolated mass disposal. Although small relative to residential contributions, these sources should be investigated to confirm their role.

It is possible that the influent, residential, and commercial mass estimates developed from flow and concentration data differ significantly from the true mass values. This is because data from a limited number of sampled sources were used to project the mass contributions for the whole population of sources. The large width of the intervals are reflective of the small sampled sizes as well as the large variability that was characteristic of the sampled sites. Increasing the sample size would increase the precision of the mass estimates. However, it is not clear whether such an improvement of data quality would be achievable at a cost-effective level. Moreover, the refinement of mass load values will not likely change the indication that residential contribution is the major component of diazinon and chlorpyrifos in the CCCSD service area. An indication of the relative importance of sources to influent loads may be sufficient in directing the source reduction strategies.

A source reduction strategy will most likely have the greatest effect if it focuses on reducing diazinon and chlorpyrifos loads from residential neighborhoods. The allotment of resources to directly reduce commercial inputs may result in limited success because of the relatively smaller commercial mass contributions. If a source reduction program is successful at increasing the pollution prevention awareness of service area residents, input from commercial and unknown sources may also indirectly decrease. However, a successful campaign must impact the specific practices that introduce diazinon and chlorpyrifos into the residential sewage. In future research, surveys and sampling of residential areas will be necessary to determine these specific practices.

The investigation of factors responsible for the lower chlorpyrifos influent load at RWQCP may also produce additional source reduction strategies for CCCSD. If they are feasible, treatment-related factors which produce greater reduction of diazinon and chlorpyrifos at RWQCP should also be considered for implementation at CCCSD. The reduction in diazinon and chlorpyrifos loads from CCCSD's influent will decrease the amount of the two active ingredients in the effluent. Adequate reduction may result in the consistent compliance with conditions under its waste discharge permit.

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Appendix A: APPL Analytical Method

Standard Operating Procedure

CDPR-STUDY 97 DIAZINON/CHLORPYRIFOS
USING SOLID PHASE EXTRACTION TECHNIQUE
ANALYSIS BY EPA METHOD 8141A[1]

STATEMENT OF PURPOSE

This procedure describes the extraction and analysis of organophosphorus pesticides, Diazinon and Chlorpyrifos, in water using a solid phase extraction technique[2].

INSTRUCTIONS

A. Scope and Application

This method is a capillary gas chromatographic method used to determine the concentration of Diazinon and Chlorpyrifos compounds in water. The fused-silica, open-tubular columns specified in this method offer improved resolution, better selectivity, increased sensitivity, and faster analysis than packed columns. A dual-column/dual-detector approach is used for the analysis of the extracts.

Table 1 lists reporting limits for the target analytes. The analyst must demonstrate chromatographic resolution of both analytes. A method validation consisting of 3 aliquots of matrix spiked at 0.25 µg/L, 3 aliquots of matrix spiked at 0.5µg/L, 3 aliquots of matrix spiked at 2.5µg/L, 10µg/L and 20µg/l was used to set control limits of 30-138% for Diazinon and 39-94% for Chlorpyrifos.

B. Method Summary

This method provides gas chromatographic conditions for the detection of ppb concentrations of Diazinon and Chlorpyrifos using a Nitrogen Phosphorus detector (NPD). The sample extracts are prepared by solid phase extraction followed by florisil cleanup as necessary.

C. Sample Preservation, Containers, Handling and Storage

Containers used to collect samples for the determination of Diazinon and Chlorpyrifos are provided by the client. The sample containers for waters are 1 liter amber glass bottles with Teflon lined screw caps.

Organophosphorus esters will hydrolyze under acidic or basic conditions. Adjust samples to a pH of 5 to 8 using sodium hydroxide or sulfuric acid solution as soon as possible after sample extraction. Record the volume used.

All samples will be taken and held at a temperature of 4°C ± 2°C until delivery to the laboratory. When the samples are delivered to the laboratory they are placed into a refrigerator that

is kept at 4°C ± 2°C until extraction. Begin sample extraction within 7 days of collection. Extracts must be stored under refrigeration and analyzed within 40 days of extraction.

D. Interferences and Potential Problems

N.A.

E. Equipment/Apparatus

1) Gas chromatograph

   A) Gas chromatograph - Hewlett Packard 5890 gas chromatograph equipped with a 7673A autosamplers or a Hewlett Packard 6890 GC equipped with 6890 autosamplers.

   B) Columns

      a) Column 1 - 30-m X 0.53-mm wide-bore capillary column, 1.5-μm film thickness, chemically bonded with 35% phenyl methyl polysiloxane (HP-35).
      b) Column 2 - 30-m X 0.53-mm wide-bore capillary column, 1.0 μm film thickness, chemically bonded with 5% phenyl polysiloxane, 95% methyl polysiloxane (DB-5).

   C) Detectors

      a) Nitrogen-Phosphorus Detector (NPD) operated in the phosphorus-specific mode.

2) Hewlett Packard EnviroQuant Data System

3) Volumetric flasks, Class A: sizes as appropriate with ground-glass stoppers.

4) Microsyringe

5) Syringes

6) Injection vials with crimp tops.

7) Balances, Analytical, 0.0001 g.

F. Reagents:

Reagent grade chemicals shall be used in all tests. Other grades may be used, provided it is first ascertained that the reagent is of sufficiently high purity to permit its use with out lessening the accuracy of the determination. All reagents used will be traceable at all steps of the procedure.

1) Solvents

    a) Hexane - Pesticide quality

    b) Acetone - Pesticide quality

    c) Methylene chloride - Pesticide quality

    d) Methanol - Pesticide quality

3) Stock standard solutions:

    a) Diazinon and Chlorpyrifos stock standards[3] are purchased as neat material. The stock standard solution is prepared by accurately weighing about 0.0100 g of pure compound. It is then dissolved in suitable mixtures of acetone and hexane and diluted to volume in a 10 mL volumetric flask. NOTE: If compound purity is 96 percent or greater, the weight can be used without correcting to calculate the concentration of the stock standard solution.

    b) The stock standard solutions are stored in the sealed ampules and are kept at 4°C ± 2°C and protected from light. Stock standards are to be checked for evaporation, especially just prior to preparing calibration standards from them.

    c) Stock standard solutions must be replaced after two months or sooner if comparison with check standards indicates a problem.

4) Calibration standards: Calibration standards at five concentrations for each parameter of interest are prepared through dilution of the stock standards with hexane. Calibration solutions must be replaced after one or two months, or sooner, if comparison with check standards indicates a problem.

5) Surrogate standards: Tributylphosphate and triphenylphosphate[4] are purchased as mixes at 1000µg/mL. A volume of 50 µL of the 1000 µg/mL is added to 10 mL of Acetone for a 5 µg/mL surrogate spiking solution. This solution is added to each sample, blank, and all QC samples. Proceed with corrective action when surrogates are out of limits for a sample.

G. Procedure

EXTRACTION

1) <u>MEASURING SAMPLE VOLUME:</u> On the side of the bottle mark the meniscus for later determination of sample volume. After the sample has been extracted, determine the sample volume by refilling the sample bottle with tap water to the mark and transferring the liquid to a 1000mL graduated cylinder. Record the sample volume to the nearest 10 mL.

2) Add 1mL of a spike mix containing Diazinon/Chlorpyrifos at 5.0 µg/mL to the QC samples. Add 0.1 mL of the surrogate mix containing Triphenylphosphate/Tributylphosphate at 5.0 µg.mL to

blanks, samples and QC samples. Filter the matrix through a GF/F Whatman filter before adding the spiking solution.

NOTE: The above step will be witnessed by a second person and will be documented on the extraction sheet.

3) Check the pH with multicolored Merck pH test strips. Adjust the pH to 5-8 if needed.

4) Assemble an all glass filtration assembly using a 90 mm C18 3M Empore ^{TM} Extraction Disk. Use of a manifold for multiple extraction is acceptable.

NOTE: If samples contains significant quantities of particulates, the use of an in-situ glass micro-fiber prefilter (Whatman GMF 150, 1 micron pore size or equivalent) is advisable. The glass fiber prefilter is placed on top of the Empore ^{TM} disk prior to placement of the glass reservoir and clamp.

5) PREWASH: Wash the extraction apparatus and disk by adding 20 ML of Methylene chloride to the reservoir, washing down the sides of the glass reservoir in the process. Pull a small amount through the disk with a vacuum: turn off the vacuum and allow the disk to soak for about one minute. Pull the remaining solvent through the disk and allow the disk to dry.

6) CONDITION: Pre-wet the disk by adding 20 ML of Methanol to the reservoir pulling a small amount through the disk, then letting it soak for about one minute. Pull most of the remaining Methanol through the disk leaving 3-5 mm on the surface of the disk, which should not be allowed to go dry from this point until the sample extraction has been completed. Should the disk accidentally dry, simply repeat the prewetting step.

7) Rinse the disk by adding 20 ML of reagent water to the disk and drawing most through, again leaving 3-5 mm of water on the surface of the disk.

8) EXTRACTION: Add the water sample to the reservoir and, adjust the vacuum to filter so that a resonance time of greater than 5 minutes is achieved. Drain as much water from the sample bottle as possible. Allow the entire sample to pass through the disk, then dry the disk by maintaining vacuum for about 3 minutes.

Note: With heavily particle laden samples, allow the sediment to settle; decant as much liquid as is practical into the reservoir. Allow most of the liquid to filter, then swirl the sediment portion and add it to the reservoir. Before the entire sample has filtered, rinse the sample bottle to the extraction. Drain as much water as possible from the sample bottle.

9) Remove the entire filter assembly (do not disassemble) and insert 20 mL amber glass vial for eluent collection. Replace filter assembly.

10) ELUTION: Add 5.0 mL Acetone to the disk. Allow the Acetone to spread evenly across the disk, then quickly turn the vacuum on and off again to pull a fraction of a milliliter through the disk. Allow the disk to soak for 15 to 20 seconds.

11) Add 20 mL of Methylene chloride to the sample bottle. Rinse the bottle thoroughly and with the Acetone still on the disk, transfer the solvent to the disk with a dispo-pipette, rinsing down the sides of the reservoir in the process. Draw about half of solvent through disk, then release the vacuum. Allow the remaining Methylene chloride to soak the disk for about one minute, then draw remainder through under vacuum.

12) Repeat the bottle rinse and elution in step 11 with a 10 mL aliquot of Methylene chloride.

13) Filter eluent through a funnel containing Sodium sulfate into a 250 mL boiling flask. Rinse Sodium sulfate with 30 mL Methylene chloride.

14) Rotovap extract down to 10 mL and transfer to 20 mL scintillation vial. Microconcentrate to dryness and bring to 1 mL final volume with Hexane.

15) If needed, perform Florisil cleanup on sample extracts according to SOP #ORG023[5]. One millileter of the extract is placed on a Superclean LC-Florisil SPE tube. The column is first eluted with 6% Ethyl ether, then 15% Ethyl ether and then 50% Ethyl ether. The eluates are microconcentrated on a rotary evaporator and the final volume is adjusted to 1 mL with Hexane.

16) Enter required If the responses exceed the linear range of the system, dilute the extract and reanalyze. It is recommended that extracts be diluted so that all peaks are on scale. Overlapping peaks are not always evident when peaks are off scale. Computer reproduction of chromatograms, manipulated to ensure all peaks are information on "Organic Extraction Worksheet" form. Deliver the properly labeled scintillation vials to GC Instrumentation Room and log into the refrigerator using the 'Organic Extracts Log-in" log book.

ANALYSIS

1) Operating Conditions (for either 5890 or 6890 GC):

GC Conditions:
     Oven Temperature: 80°C
     Equib Time: 1.00 min
     Oven Maximum: 290°C
     Initial Temp: 80°C
     Initial Time: 0.00 min

Temperature Program:
     Rate: 10.0°C
     Final Temp: 290°C
     Final Time: 9.0 min

Injection Temp: 225°C
Detector Temp: 290°C

2) Calibration: Prior to using this introduction technique for any GC method, the system is calibrated.

a) Calibration standards at five concentrations for each parameter of interest are prepared through dilution of the stock standards with hexane. The concentrations are: 0.035, 0.105, 0.175, 0.525, and 0.700 µg/mL. A mid point of 0.250 µg/mL is injected every 10 injections.

b) Calibration solutions must be replaced after two months, or sooner, if comparison with check standards indicates a problem.

c) Analyze each calibration standard. Inject each calibration standard using the technique that will be used to introduce the actual samples into the gas chromatograph. Samples are introduced into the gas chromatograph using a syringe, the ratio of the response to the amount injected, defined as the calibration factor (CF), can be calculated for each analyte at each standard concentration. If the percent relative standard deviation (%RSD) of the calibration factor is less than 20% over the working range, linearity through the origin can be assumed, and the average calibration factor can be used in place of a calibration curve.

d) Calculate response factors or calib ration factors for each analyte of interest as follows:

$$\text{Calibration Factor} = \frac{\text{Total Area of Peak}}{\text{Mass injected (in nanograms)}}$$

e) The working calibration factor must be verified on each working day by the injection of one or more calibration standards. The frequency of verification is every 10 injections. If the response for any analyte varies from the predicted response by more than $\pm 15\%$, a new calibration curve must be prepared for that analyte.

$$\text{Percent Difference} = \frac{R_1 - R_2}{R_1} \times 100$$

where:
R_1 = Average calibration factor from first analysis
R_2 = Calibration factor from continuing analysis

3) Gas chromatographic analysis:

a) Diazinon and Chlorpyrifos compounds are introduced by direct injection using HP autosamplers.

b) Samples are analyzed in a set referred to as an analysis sequence. The Sequence begins with the instrument calibration followed by sample extracts, and, every 10

injections a mid level calibration standard mix. The sequence ends when the set of samples has been injected or when qualitative and or quantitative QC criteria are exceeded.

c) If the responses exceed the linear range of the system, dilute the extract and reanalyze. It is recommended that extracts be diluted so that all peaks are on scale. Overlapping peaks are not always evident when peaks are off scale. Computer repro0duction of chromatograms, manipulated to ensure all peaks are on scale over a 100 fold range, are acceptable if linearity is demonstrated.

d) Calibrate the system immediately prior to conducting any analyses. The calibration factor for each analyte to be quantitated, must not exceed 15% difference. When this criterion is exceeded, inspect the GC system to determine the cause and perform whatever maintenance is necessary before recalibrating and proceeding with sample analysis. All samples that were injected after the standard exceeding the criterion must be reinjected to avoid errors in quantitation, if the initial analysis indicated the presence of specific target analytes that exceeded the criterion.

e) Establish daily retention time windows [6] for each analyte. Use the retention time for each analyte as the midpoint of the window for that day.
  1) The daily retention time window equals the midpoint $\pm$ three times the standard deviation determined from standards injected over a 72 hour period.

  2) In those cases where the standard deviation for a particular standard is zero, substitute the standard deviation of a close eluting, similar compound to develop a valid retention time window.

  3) A new retention time study is completed annually or whenever a new column is installed.

f) Tentative identification of an analyte occurs when a peak from a sample extract falls within the daily retention time window. Normally, confirmation is required on a second GC column. Confirmation may not be necessary if the composition of the sample matrix is well established by prior analyses.

g) Validation of the GC system qualitative performance: Use the mid concentration standards every 10 injections throughout the analysis sequence to evaluate this criterion.

h) Refer to Section (H) Calculations (below) for guidance on calculating of concentration.

i) Using external calibration procedure, determine the identity and quantity of each component peak in the sample chromatogram which corresponds to the compounds used for calibration purposes.

j) If peak detection and identification are prevented due to interferences, the hexane extract may need to undergo cleanup using Florisil clean up as described above in extraction. The resultant extract(s) are analyzed by GC.

H. Calculations

1) The sample/spike/surrogate is calculated against the initial calibration curve.

2) External standard calibration: The quantitation of each analyte is performed by the EnviroQuant Data System. The algorithm is checked at least once per computer file (daily) by calculating the amount of analyte injected, from the peak response, using the calibration curve or the calibration factor. The following calculation is used to check the quantitation:

$$\text{Concentration } (\mu g/L) = \frac{(A_s)(C_{st})(V_e)}{(A_{st})(V_o)(1000)} \times D$$

where:

A_s = Response for the analyte to be measured, (area or height)
A_{st} = Response for the external standard
C_{st} = Concentration of external standard ($\mu g/mL$)
V_o = Volume of sample extracted (L)
V_e = Final volume of extract (mL)
D = Dilution factor, if a dilution was made

I. Quality Control

1) The laboratory must demonstrate initial proficiency with each sample preparation and determinative method combination it utilizes, by generating data of acceptable accuracy and precision for target analytes in a clean matrix.

2) Calibration Criteria: The relative percent difference (%RSD) for the initial calibration curve must be ≤ 20% to validate the sequence. Include a calibration standard after each group of 10 samples in the analysis sequence as a calibration check. The response factor of the continuing calibration check must be ≤ 15% from the response factor in the initial calibration curve. When this continuing calibration is out of this acceptance window, the laboratory will stop analyses and take corrective action.

3) Sample quality control for preparation and analysis include the analysis of a method blank, a matrix spike/matrix spike duplicate, and laboratory control sample in each analytical batch and the addition of surrogates to each sample and QC sample.

4) A matrix spike/matrix spike duplicate (MS/MSD) will be included with each batch of 20 samples or less. The MS/MSD will contain the analytes of interest at the following concentrations:

> Diazinon at 5.0µg/mL
> Chlorpyrifos at 5.0µg/mL

1 mL of the above concentration is added to approximately 1000 mL of the sample selected for the matrix spike QC. The matrix is filtered through a G5/5 Whatman before addition of the spiking solution.

5) A laboratory control sample (LCS) will be included with each batch of 20 samples or less. The LCS consists of an aliquot of control matrix similar to the sample matrix and of the sample weight or volume. The LCS is spiked with the same analytes at the same concentrations as the MS/MSD. When the results of the matrix spike analysis indicate a potential problem due to the sample matrix itself, the LCS results are used to verify that the laboratory can perform the analysis in a clean matrix.

6) The surrogate solution, Triphenylphosphate/Tributylphosphate at 5.0 µg/mL each, is added to blank, samples and QC samples.

7) If recovery is not within limits for either the spiked sample recovery or surrogate recovery, the following are required:

   a) Confirm that there are no errors in calculations, surrogate solutions and internal standards. Also, check instrument performance.
   b) Examine chromatograms for interfering peaks and for integrated areas.
   c) Recalculate the data and/or reanalyze the extract. Many of the above checks reveal a problem.
   d) Reextract and reanalyze the sample if none of the above are a problem or flag the data as "estimated concentration".

8) Method Detection Limit[7]: To seven aliquots of water, 1 mL of a 0.25 µg/mL Diazinon and Chlorpyrifos concentration mix is added. The seven samples are extracted and analyzed and the MDL is calculated by the following equation:

$$MDL = (t)(S)$$

where:

> t = the student t value appropriate for a 99% confidence level (for 7 replicates, $t = 3.143$).

> S = standard deviation of the replicate analyses.

9) PQL: The practical quantitation limit is established by multiplying the MDL obtained above by 1 to 5 times. It is recognized that the experience of the analyst is important to this procedure. The PQL for Diazinon and Chlorpyrifos is 1.25 times the MDL.

10) Deviations: Any activity not performed in accordance with laboratory procedures or Quality Assurance Project Plans is considered a deviation from the plan. All deviations from plan will be documented as to the extent of, and reason for, the deviation.

11) Corrective Action: Errors, deficiencies, deviations, or laboratory events or data that fall outside of established acceptance criteria will be investigated. In some instances, corrective action may be needed to resolve the problem and restore proper functioning to the analytical system. The investigation of the problem and any subsequent corrective action taken is documented on a Quality Control Exception Report (QCER) and/or a Corrective Action Report (CAR).

12) GC/MS confirmation: Any compounds confirmed by two columns may also be confirmed by GC/MS if the concentration is sufficient for detection by GC/MS as determined by the laboratory generated detection limits. The extract and the associated blank will be analyzed.

J. Data Validation

Data is first reviewed by the analyst completing the work. The initial calibration curve is reviewed, the continuing calibration %D is reviewed and the spike recovery and precision are reviewed. If at any point the review shows an out of control situation the section manager is notified verbally and the problem is investigated. The correction may be one of several points considered: standard preparation, improper injection size, extraction technique, etc. The problem is potentially solved and reanalyses are completed.

The second level of review is either by a peer in the same section or the section manager. There is a Multilevel Quality Control Sign Off worksheet that is filled out in its entirety by the review person.

When QC parameters are exceeded, the following will take place: When the matrix spikes are outside of the limits they are redigested and reanlyzed. When the LCS is outside of limits the entire batch is reextracted and reanalyzed. If there is not enough sample for reextraction the Project Manager is notified who in turn notifies the client by phone or fax. The case narrative or case letter explains the sequence of events and the data is qualified. If the calibration parameters are not met the standards are reprepared and reanalyzed.

K. Health and Safety

Lab coats and gloves are worn at all times. All personnel handling raw samples must have been vaccinated or tittered for infectious diseases. Follow all safety procedures as described in the SOP for samples suspected of containing biological hazards.

Standard Operating Procedure
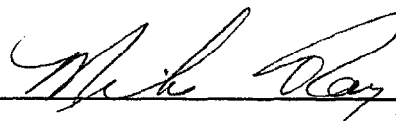
SALUTATION

This procedure applies to all personnel who extract and analyze samples for Diazinon and Chlorpyrifos by solid phase extraction technique[2].
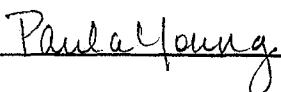
Agriculture & Priority Pollutants Laboratories, Inc.
4203 West Swift Avenue
Fresno, California 93722
(209)-275-2175
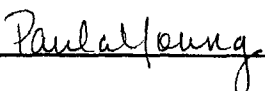
Section Manager: _____ Date: 9-22-98

Laboratory Director: _____ Date: 09/22/98

QAU Director: _____Paula Young_____ Date: 9/22/98

Prepared by: _____Paula Young_____ Date: 9/22/98

References:

1 EPA Method 8141A, USEPA SW846, Third Edition Revision 1 September 1994

2 APPL SOP #MWE009 Revision 1 May 28, 1996

3 Chem Service

4 Protocol

5 APPL SOP #ORG023 Revision 2 February 6, 1996 (EPA Method 3620A, USEPA SW846, Third Edition Revision 1 July 1992)

6 EPA Method 8000A, USEPA SW-846, Third Edition, Revision 1, July 1992

7 CFR 40 Part 136, Appendix B, Chapter 1 (7-1-95 Edition), Revision 1.11

TABLE 1

STUDY 97 COMPOUNDS

Compound	Water Quatitation Limit (µg/L)	Soil Quantitation Limit (mg/Kg)
Diazinon	0.05	NA
Chlorpyrifos	0.05	NA

Appendix B: EHAP Standard Operating Procedures (SOP QAQC.001.00)

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Chemistry Laboratory Quality Control

KEY WORDS

QC; method detection limit; MDL; reporting limit; RL; confirmation; verification; AB 2021; method development; method validation; storage stability; split; spike; blank; laboratory specifications

APPROVALS

APPROVED BY:_____ DATE: 7/31/95
                    Management

APPROVED BY:_____ DATE: 7/31/95
                    EHAP Senior Scientist

APPROVED BY:_____ DATE: 7/28/95
                    EHAP Quality Assurance Officer

PREPARED BY:_____ DATE: 7/28/95

Environmental Hazards Assessment Program (EHAP) organization and personnel such as management, senior scientist, quality assurance officer, project leader, etc. are defined and discussed in SOP ADMN002.

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STANDARD OPERATING PROCEDURE
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1 .O INTRODUCTION

1 .1 Purpose

This Standard Operating Procedure (SOP) discusses the chemistry laboratory quality control (QC). These guidelines describe method development as well as continuing quality control procedures that should be followed for all Environmental Hazards Assessment Program (EHAP) studies.

1.2 Definitions

1.2.1 **AB 2021 Confirmation** refers to the detection of a pesticide in at least two discrete well samples.

1.2.2 **AB 2021 Verification** refers to analysis "by a second analytical method or a second analytical laboratory approved by the department." Confirmation and verification are defined and discussed at length (particularly in the AB 2021 context) in the memorandum from Randy Segawa to Kean Goh, dated 1 l/22/93,

1.2.3 Analytical Confirmation refers to an analyte that has been unequivocally identified. For an analytical method that is nonspecific (e.g., gas chromatography with a flame photometric detector) analytical confirmation requires a second analysis that has a change in both the separation and detection principle. Except for AB 2021 projects, an analytical method that is specific (e.g., mass spectrometry) meets the analytical confirmation criterion and a second analysis is not required. AB 2021 requires a second analysis even if the primary method is specific.

1.2.4 **Blank** refers to a sample with no detectable amount of pesticide. Blanks are used to check for contamination or to prepare QC samples (e.g., **blank-matrix, reagent. blank,** and **field blank** samples).

1.2.5 **Blind Spike** refers to a blank-matrix sample which has been spiked and submitted to the lab disguised as a field sample.

1.2.6 **Extract** refers to the final solvent which contains the pesticide residue.

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1.2.7 **Extraction Set** refers to a single group of samples extracted and processed at the same time.

1.2.8 **Instrument Detection Limit (IDL)** is 1 - 5 times the signal-to-noise ratio depending on the analytical method.

1.2.9 **Method Detection Limit (MDL)** refers to the USEPA definition (40 CFR, Part 136, Appendix B). "The MDL is defined as the minimum concentration of a substance that can be measured and reported with 99% confidence that the analyte concentration is greater than zero and is determined from analysis of a sample in a given matrix...."

1.2.10 Reporting Limit (RL) is 1 - 5 times the MDL depending on the analytical method and matrix. The MDL can vary from sample to sample because of matrix effects. Ideally, the RL will not change, will be set high enough to account for matrix effects, yet low enough to be useful.

1.2.11 **Spike** refers to a known amount of pesticide added. These QC samples are used to check the precision and accuracy of a method.

1.2.12 **Split** refers to one homogeneous sample divided into several aliquots, with the different aliquots analyzed by different Jaboratories. These QC samples are used to check the specificity and precision of a method.

1.2.13 **Standard** refers to the laboratory analytical standard.

2.0 GENERAL PROCEDURES

These guidelines are meant to be a starting point; a specific study may require more or less QC than is given here. The procedures outlined here are the QC measures which should be reported. Performing other QC procedures such as frequency of standard injections and calibrations are left to the chemist's discretion.

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2.1 General Method Development

Many times the method development will be a negotiation between the project
leader and the laboratory. The project leader can suggest some method
perfomance goals (e.g., specificity, reporting limit, etc.), butthe goals need to be
balanced with laboratory cost and time constraints. The, method performance
should be consistent with the study objectives.

> 2.1 .l   Standard - Standard solutions should be validated prior to use by
> checking for chromatographic purity or verification of the concentration using
> a second standard prepared at a different time or obtained from a different
> source.
>
> 2.1.2 Method Defection Limit Determination - The MDL is determined by the
> USEPA method (40 CFR, Part 136, Appendix B). The complete procedure is
> given in Appendix I, Briefly, the MDL is' determined by analyzing at least 7
> low-level matrix spikes (generally 1 - 5 times the IDL) and performing the
> following calculation:

$$MDL = t \times S$$

> where:
> > t = Student's t value for 99% confidence level (l-tailed) and n-l
> > degrees of freedom
> > S = standard deviation
>
> *2.1.3 Reporting Limit Determination* - The RL is determined by the chemist
> and set at 1 - 5 times the MDL depending on the matrix and instrument.
>
> *2.1.4 Method Validation* - At the onset of a study, an acceptable range of
> spike recoveries will be established. This range will be established by
> analyzing blank-matrix spike samples. Two to five replicate analyses at two
> to five different spjke levels will be used to determine the mean percent
> recovery and standard deviation. Number of replicates and spike levels will
> be chosen by the project leader. Warning limits will be established at the
> mean percent recovery plus/minus 1 - 2 times the standard deviation.
> Control limits will be established at the mean percent recovery plus/minus 2 -

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Chemistry Laboratory Quality Control

___

3 times the standard deviation. Any subsequent spiked samples'outside the control limits may require the set of samples associated with that spike to be reanalyzed.

2.1.5 Storage Stability - Storage stability needs to be evaluated on a case-by-case basis, so no specific test design is specified. H'owever, in general the test should be run for the longest anticipated holding period, with at least four sampling intervals and two replicate samples at each sampling interval. Other factors may also need to be incorporated into the storage stability tests, such as pH, temperature, and container type. The project leader is responsible for specifying the design of the storage stability test.

2.2 General Continuing QC - These analyses are to be done- by the main lab on a continuing basis. Each extraction set should consist of 5-20 actual samples. Exact frequency of QC analyses and spike levels are chosen by the project leader.

2.2.1 *Reagent* Blanks - 1 - 2 per extraction set

2.2.2 *Blank-Matrix* Spikes - 1 - 3 per extraction set

2.2.3 Analytical Confirmafion - 0 to 100% (normally 10%) of positive samples confirmed

2.2.4 *Split Matrix Samples* - 0 to 100% (normally 10%) of the actual samples should be split into two aliquots, one aliquot analyzed by the main lab, and one by the QC lab. For studies that cannot have actual samples split or for which only a few positives are anticipated, blind spike samples may be used.

2.2.5 Blind Spikes - 0 to 100% (normally 10%) of the actual samples should be accompanied by laboratory-spiked samples disguised as real samples. These should be done only for matrices that can be accurately spiked.

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2.3 Optional Continuing QC - The following analyses should be considered but may not be routinely performed unless specified by the project leader.

2.3.1 Infernal Standard - a chemical not expected in the samples can be spiked into all samples or extracts. This is particularly useful for quantifying mass spectrometry data.

2.3.2 Replicate Sample Analyses - analyzing multiple aliquots of a single sample will give a bettter estimate of the method precision.

2.3.3 Replicate Exfracf Analyses - multiple analyses of a single extract will give a separate estimate of the precision of the extraction and analysis processes.

2.3.4 Split Exfracf Analyses - analyzing a single extract with more than one lab is useful for checking discrepancies between laboratories.

2.3.5 Reference Material - a stable sample that contains the analyte(s) of interest and has been analyzed many times so that the concentration(s) are known. Analysis of this material may give a better estimate of the method's accuracy than spiked samples. Also useful for method development.

2.3.6 Standards Exchange - exchanging analytical standards between the primary and QC lab is useful for checking discrepancies in split samples.

3.0 WELL WATER STUDY QC PROCEDURES

3.1 Well Water Study Method Development - The general method development procedures should be used.

3.2 We'll Water Study Continuing QC - The following specific continuing QC should be used in place of the general continuing QC:

3.2.1 Reagent Blanks - 1 to 2 per extraction set

3.2.2 Blank-Matrix Spikes - 1 to 3 per extraction set

STANDARD OPERATING PROCEDURE
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3.2.3 AB 2027 confirmation and verification - at least one additional sample from the same well <u>must</u> be analyzed by a second lab or a second method for each positive sample. AB 2021 confirmation requires positive detection in at least 2 discrete samples and verification with a second lab or a second method.

3.2.4 Blind Spikes - 1 blind spike should be submitted for every 3 - 50 well samples.

3.2.5 Field Blanks - 1 field blank should be collected at each well, but analyzed only if the well sample is positive.

4.0 AIR STUDY QC PROCEDURES

4.1 Air Study Method Validation (trapping efficiency) - In addition to the general procedures, the trapping efficiency should be determined. This normally involves collecting a series of 2-stage air samples. The top stage sampling tube contains glass-wool and is spiked. The bottom stage consists of the normal sampling tube. The 2-stage sample is placed on an air sampler and run for the appropriate amount of time. Both stages are then analyzed to determine the proportion of the spike trapped in the bottom stage. The test should consist of two to five replicate analyses at two to five spike levels. Samplers should run for various lengths of time, if necessary. To determine the precision of the spiking technique, five sample tubes with glass wool should be spiked and analyzed. Oxidation products should also be analyzed to determine the rate of conversion. Exact test specifications are chosen by the project leader.

4.2 Air Study Continuing QC - In addition to the general procedures, one reagent spike should be analyzed with each extraction set. The air sampling matrix will occasionally give an enhanced detector response.

In general, it is not possible to split air samples, so split matrix analyses are not usually done.

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5.0 CALCULATIONS

5.1 Calculating the Method Detection Limit - The MDL is determined by
performing the following calculation:

$$MDL = t \times S$$

where:

> t = Student's t value for 99% confidence level (l-tailed) and n-l degrees of
> freedom
> S = standard deviation

5.2 Calculating Warning and Control Limits - The method validation data are
used to set warning and control limits. Warning limits will be established at the
mean percent recovery plus/minus 1 - 2 times the standard deviation. Control limits
will be established at the mean percent recovery plus/minus 2 - 3 times the standard
deviation. Any subsequent spiked samples outside the control limits may require
the set of samples associated with that spike to be reanalyzed.

6.0 REPORTING REQUIREMENTS

These reporting requirements pertain only to the QC data. There may be other
reporting requirements specified in the EHAP Analytical Laboratory Specifications
Form (Appendix 2).

6.1 Reporting Method Development Results - The following should be reported
by the lab to the EHAP QA officer prior to the start of any field sample analyses: the
spike level and concentration detected for each sample of the MDL determination,
the method validation, and the storage stability. The EHAP QA officer will review,
summarize and submit the data to the project leader.

6.2 Reporting Continuing QC Results - The following QC results should be
reported by the lab to the EHAP QA officer on a continuous basis: the concentration
of all blanks, the concentration detected for all spikes, the amount added for all
spikes. Any spiked samples outside the control limits may require the set of
samples associated with that spike to be reanalyzed, The EHAP QA officer will

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review, summarize and submit the data to the project leader. In addition, the project leader may request to be notified if any problems arise during,the course of chemical analysis.

6.3 Reporting Sample Results - The laboratory should not use any spike or blank data to adjust the **field** sample results, unless specified by the project leader. Any adjustments should be made by EHAP personnel.

7.0 STUDY-SPECIFIC DECISIONS

The project leader is responsible for the following specific decisions for each individual study. These decisions must be made for both the primary lab and the QC lab, if one is used. All decisions should be given to the EHAP QA officer who will document the decisions and transmit them to the lab using the EHAP Analytical Laboratory Specifications Form.

7.1 Method performance goals - reporting limit, specificity, precision, accuracy, sample size, time to complete analysis, etc.

7.2 Number of MDL spike samples

7.3 Method validation spike levels and number of replicates

7.4 Warning and control limit criteria (1 - 3X standard deviation)

7.5 Storage stability test design

7.6 Number or frequency of continuous QC spike analyses

7.7 Concentration of continuous QC spike samples

7.8 Number or frequency of analytical confirmation

7.9 Number or frequency of split analyses

7.10 Use, selection and concentration of an internal standard

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7.11 Number or frequency of replicate sample analyses

7.12 Number or frequency of blind spike analyses

7.13 Concentration of blind spike samples (also select analyte(s) if multi-residue method)

7.14 Number or frequency of replicate extract analyses

7.15 Number or frequency of split extract analyses

7.16 Number or frequency of standard reference material analyses

7.17 Method of AB 2021 verification - 2nd lab or 2nd method

7.18 Trapping efficiency test design

7.19 Number or frequency of reagent spike analyses

8.0 REFERENCES

California Department of Pesticide Regulation. 1988. Chemistry Laboratory Quality Control Guidelines. Environmental Hazards Assessment Program.

Segawa, R. 1993. AB 2021 Confirmation and Verification Policy. Memorandum to Kean Goh, dated November 22, 1993. Environmental Hazards Assessment Program.

APPENDIX 1 - U.S. EPA Method Detection Limit Determination

APPENDIX 2 - Analytical Laboratory Specifications

APPENDIX 1

APPENDIX B TO PART 136—DEFINITION AND PROCEDURE FOR THE DETERMINATION OF THE METHOD DETECTION LIMIT—REVISION 1.11

Definition

The method detection limit (MDL) is defined as the minimum concentration of a substance that can be measured and reported with 99% confidence that the analyte concentration is greater than zero and is determined from analysis of a sample in a given matrix containing the analyte.

Scope and Application

This procedure is designed for applicability to a wide variety of sample types ranging from reagent (blank) water containing analyte to wastewater containing analyte. The MDL for an analytical procedure may vary as a function of sample type. The procedure requires a complete, specific, and well defined analytical method. It is essential that all sample processing steps of the analytical method be included in the determination of the method detection limit.

The MDL obtained by this procedure is used to judge the significance of a single measurement of a future sample.

The MDL procedure was designed for applicability to a broad variety of physical and chemical methods. To accomplish this, the procedure was made device- or instrument-independent.

Procedure

1. Make an estimate of the detection limit using one of the following:

(a) The concentration value that corresponds to an instrument signal/noise in the range of 2.5 to 5.

(b) The concentration equivalent of three times the standard deviation of replicate instrumental measurements of the analyte in reagent water.

(c) That region of the standard curve where there is a significant change in sensitivity, i.e., a break in the slope of the standard curve.

(d) Instrumental limitations.

It is recognized that the experience of the analyst is important to this process. However, the analyst must include the above considerations in the initial estimate of the detection limit.

2. Prepare reagent (blank) water that is as free of analyte as possible. Reagent or interference free water is defined as a water sample in which analyte and interferent concentrations are not detected at the method detection limit of each analyte of interest. Interferences are defined as systematic errors in the measured analytical signal of an established procedure caused by the presence of interfering species (interferent). The interferent concentration is presupposed to be normally distributed in representative samples of a given matrix.

3. (a) If the MDL is to be determined in reagent (blank) water, prepare a laboratory standard (analyte in reagent water) at a concentration which is at least equal to or in the same concentration range as the estimated method detection limit. (Recommend between 1 and 5 times the estimated method detection limit.) Proceed to Step 4.

(b) If the MDL is to be determined in another sample matrix, analyze the sample. If the measured level of the analyte is in the recommended range of one to five times the estimated detection limit, proceed to Step 4.

If the measured level of analyte is less than the estimated detection limit, add a known amount of analyte to bring the level of analyte between one and five times the estimated detection limit.

If the measured level of analyte is greater than five times the estimated detection limit, there are two options.

(1) Obtain another sample with a lower level of analyte in the same matrix if possible.

(2) The sample may be used as is for determining the method detection limit if the analyte level does not exceed 10 times the MDL of the analyte in reagent water. The variance of the analytical method changes as the analyte concentration increases from the MDL, hence the MDL determined under these circumstances may not truly reflect method variance at lower analyte concentrations.

4. (a) Take a minimum of seven aliquots of the sample to be used to calculate the method detection limit and process each through the entire analytical method. Make all computations according to the defined method with final results in the method reporting units. If a blank measurement is required to calculate the measured level of analyte, obtain a separate blank measurement for each sample aliquot analyzed. The average blank measurement is subtracted from the respective sample measurements.

(b) It may be economically and technically desirable to evaluate the estimated method detection limit before proceeding with 4a. This will: (1) Prevent repeating this entire procedure when the costs of analyses are high and (2) insure that the procedure is being conducted at the correct concentration. It is quite possible that an inflated MDL will be calculated from data obtained at many times the real MDL even though the level of analyte is less than five times the calculated method detection limit. To insure that the estimate of the method detection limit is a good estimate, it is necessary to determine that a lower concentration of analyte will not result in a signifi-

cantly lower method detection limit. Take two aliquots of the sample to be used to calculate the method detection limit and process each through the entire method, including blank measurements as described above in 4a. Evaluate these data:

(1) If these measurements indicate the sample is in desirable range for determination of the MDL, take five additional aliquots and proceed. Use all seven measurements for calculation of the MDL.

(2) If these measurements indicate the sample is not in correct range, reestimate the MDL, obtain new sample as in 3 and repeat either 4a or 4b.

5. Calculate the variance (S^2) and standard deviation (S) of the replicate measurements, as follows:

$$S^2 = \frac{1}{n-1}\left[\sum_{i=1}^{n}X_i^2-\left(\sum_{i=1}^{n}X_i\right)^2 \Big/ n\right]$$

$$S=(S^2)^{1/2}$$

where:

X_i; i=1 to n, are the analytical results in the final method reporting units obtained from the n sample aliquots and Σ refers to the sum of the X values from i=1 to n.

6. (a) Compute the MDL as follows:

$$MDL = t_{(n-1, 1-\alpha = 0.99)}\ (S)$$

where:

MDL = the method detection limit

$t_{(n-1, 1-\alpha = .99)}$ = the students' t value appropriate for a 99% confidence level and a standard deviation estimate with n-1 degrees of freedom. See Table.

S = standard deviation of the replicate analyses.

(b) The 95% confidence interval estimates for the MDL derived in 6a are computed according to the following equations derived from percentiles of the chi square over degrees of freedom distribution (χ^2/df).

LCL = 0.64 MDL

UCL = 2.20 MDL

where: LCL and UCL are the lower and upper 95% confidence limits respectively based on seven aliquots.

7. Optional iterative procedure to verify the reasonableness of the estimate of the MDL and subsequent MDL determinations.

(a) If this is the initial attempt to compute MDL based on the estimate of MDL formulated in Step 1, take the MDL as calculated in Step 6, spike the matrix at this calculated MDL and proceed through the procedure starting with Step 4.

(b) If this is the second or later iteration of the MDL calculation, use S^2 from the current MDL calculation and S^2 from the previous MDL calculation to compute the F-ratio. The F-ratio is calculated by substituting the larger S^2 into the numerator S^2_A and the other into the denominator S^2_B. The computed F-ratio is then compared with the F-ratio found in the table which is 3.05 as follows: if $S^2_A/S^2_B<3.05$, then compute the pooled standard deviation by the following equation:

$$S_{pooled} = \left[\frac{6S^2_A+6S^2_B}{12}\right]^{1/2}$$

if $S^2_A/S^2_B>3.05$, respike at the most recent calculated MDL and process the samples through the procedure starting with Step 4. If the most recent calculated MDL does not permit qualitative identification when samples are spiked at that level, report the MDL as a concentration between the current and previous MDL which permits qualitative identification.

(c) Use the S_{pooled} as calculated in 7b to compute the final MDL according to the following equation:

$$MDL=2.681\ (S_{pooled})$$

where 2.681 is equal to $t_{(12, 1-\alpha = .99)}$.

(d) The 95% confidence limits for MDL derived in 7c are computed according to the following equations derived from precentiles of the chi squared over degrees of freedom distribution.

LCL=0.72 MDL

UCL=1.65 MDL

where LCL and UCL are the lower and upper 95% confidence limits respectively based on 14 aliquots.

TABLES OF STUDENTS' t VALUES AT THE 99 PERCENT CONFIDENCE LEVEL

Number of replicates	Degrees of freedom (n-1)	$t_{(n-1, .99)}$
7	6	3.143
8	7	2.998
9	8	2.896
10	9	2.821
11	10	2.764
16	15	2.602
21	20	2.528
26	25	2.485
31	30	2.457
61	60	2.390
∞	∞	2.326

Reporting

The analytical method used must be specifically identified by number or title and the MDL for each analyte expressed in the appropriate method reporting units. If the analytical method permits options which

affect the method detection limit, these conditions must be specified with the MDL value. The sample matrix used to determine the MDL must also be identified with MDL value. Report the mean analyte level with the MDL and indicate if the MDL procedure was iterated. If a laboratory standard or a sample that contained a known amount analyte was used for this determination, also report the mean recovery.

If the level of analyte in the sample was below the determined MDL or exceeds 10 times the MDL of the analyte in reagent water, do not report a value for the MDL.

[49 FR 43430, Oct. 26, 1984; 50 FR 694, 696, Jan. 4, 1985, as amended at 51 FR 23703, June 30, 1986]

APPENDIX C TO PART 136—INDUCTIVELY COUPLED PLASMA—ATOMIC EMISSION SPECTROMETRIC METHOD FOR TRACE ELEMENT ANALYSIS OF WATER AND WASTES METHOD 200.7

1. Scope and Application

1.1 This method may be used for the determination of dissolved, suspended, or total elements in drinking water, surface water, and domestic and industrial wastewaters.

1.2 Dissolved elements are determined in filtered and acidified samples. Appropriate steps must be taken in all analyses to ensure that potential interferences are taken into account. This is especially true when dissolved solids exceed 1500 mg/L. (See Section 5.)

1.3 Total elements are determined after appropriate digestion procedures are performed. Since digestion techniques increase the dissolved solids content of the samples, appropriate steps must be taken to correct for potential interference effects. (See Section 5.)

1.4 Table 1 lists elements for which this method applies along with recommended wavelengths and typical estimated instrumental detection limits using conventional pneumatic nebulization. Actual working detection limits are sample dependent and as the sample matrix varies, these concentrations may also vary. In time, other elements may be added as more information becomes available and as required.

1.5 Because of the differences between various makes and models of satisfactory instruments, no detailed instrumental operating instructions can be provided. Instead, the analyst is referred to the instruction provided by the manufacturer of the particular instrument.

2. Summary of Method

2.1 The method describes a technique for the simultaneous or sequential multiele-ment determination of trace elements in solution. The basis of the method is the measurement of atomic emission by an optical spectroscopic technique. Samples are nebulized and the aerosol that is produced is transported to the plasma torch where excitation occurs. Characteristic atomic-line emission spectra are produced by a radiofrequency inductively coupled plasma (ICP). The spectra are dispersed by a grating spectrometer and the intensities of the lines are monitored by photomultiplier tubes. The photocurrents from the photomultiplier tubes are processed and controlled by a computer system. A background correction technique is required to compensate for variable background contribution to the determination of trace elements. Background must be measured adjacent to analyte lines on samples during analysis. The position selected for the background intensity measurement, on either or both sides of the analytical line, will be determined by the complexity of the spectrum adjacent to the analyte line. The position used must be free of spectral interference and reflect the same change in background intensity as occurs at the analyte wavelength measured. Background correction is not required in cases of line broadening where a background correction measurement would actually degrade the analytical result. The possibility of additional interferences named in 5.1 (and tests for their presence as described in 5.2) should also be recognized and appropriate corrections made.

3. Definitions

3.1 *Dissolved*—Those elements which will pass through a 0.45 μm membrane filter.

3.2 *Suspended*—Those elements which are retained by a 0.45 μm membrane filter.

3.3 *Total*—The concentration determined on an unfiltered sample following vigorous digestion (Section 9.3), or the sum of the dissolved plus suspended concentrations. (Section 9.1 plus 9.2).

3.4 *Total recoverable*—The concentration determined on an unfiltered sample following treatment with hot, dilute mineral acid (Section 9.4).

3.5 *Instrumental detection limit*—The concentration equivalent to a signal, due to the analyte, which is equal to three times the standard deviation of a series of ten replicate measurements of a reagent blank signal at the same wavelength.

3.6 *Sensitivity*—The slope of the analytical curve, i.e. functional relationship between emission intensity and concentration.

3.7 *Instrument check standard*—A multielement standard of known concentrations prepared by the analyst to monitor and verify instrument performance on a daily basis. (See 7.6.1)

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ANALYTICAL LABORATORY SPECIFICATIONS

Project No._____   Lab   _____
Lab Project Manager _____   Phone   _____
Project Chemist _____   Phone   _____
EHAP Project Manager_____   Phone   _____
EHAP Lab Liaison/ QA Officer   Nancy Miller   Phone   322-3082

Type of Analysis:

	Sample Type	**Analysis For**	**Reporting Limit**	**Number of Samples**
1				
2				
3				
4				

Methods Development: See attachment
Sample Storage: _____
Sample Storage: _____
Sample Extraction: _____
Analytical Standard Source: _____
Instrumentation: _____
Confirmation Method: _____
Continuing QC: See attachment
Sample Disposition: _____
Extract Disposition: _____
Reporting/Turnaround: See attachment
Cost of Analysis: See attachment

Other Specifications:

_____
_____
_____
_____
_____
_____
_____

Approved by: Nancy Miller_____   _____   _____
                 CDPR Representative   Lab Representative   Date

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ANALYTICAL LABORATORY SPECIFICATIONS

METHODS DEVELOPMENT AND VALIDATION

Specifications   **Validation***

Method # _____
Sample Matrix: _____   **Sample Type**   **Spike Level**   **# Reps**
Analyzed For: _____   1
Reporting Limit: _____   2
Other Specifications: _____ 3
_____   4
_____   5
_____
_____

Method # _____
Sample Matrix: _____   **Sample Type**   **Spike Level**   **# Reps**
Analyzed For: _____   1
Reporting Limit: _____   2
Other Specifications: _____ 3
_____   4
_____   5
_____
_____

Method # _____
Sample Matrix: _____   **Sample Type**   **Spike Level**   **# Reps**
Analyzed For: _____   1
Reporting Limit: _____   2
Other Specifications: _____ 3
_____   4
_____   5
_____
_____

* Each laboratory shall determine a method detection limit (MDL), instrument detection limit (IDL) and a
reporting limit (RL) for each analyte. Each laboratory shall also document their terms, definitions and
procedures for determining MDL, IDL and RL in their approved analytical method. Each laboratory
shall provide a copy of their approved analytical method before analyzing any field samples.
The results from the method validation study will be used to establish recovery control limits for the field study.

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METHODS DEVELOPMENT

Specifications   **Validation***

Method # _____

Sample Matrix: _____   **Sample Type**   **Spike Level**   **# Reps**

Analyzed For: _____   1

Reporting Limit: _____   2

Other Specifications: _____   3

_____   4

_____   5

_____

_____

Method # _____

Sample Matrix: _____   **Sample Type**   **Spike Level**   **# Reps**

Analyzed For: _____   1

Reporting Limit: _____   2

ner Specifications: _____   3

_____   4

_____   5

_____

_____

Method # _____

Sample Matrix: _____   **Sample Type**   **Spike Level**   **# Reps**

Analyzed For: _____   1

Reporting Limit: _____   2

Other Specifications: _____   3

_____   4

_____   5

_____

_____

* The results from the method validation study will be used to establish recovery control limits for the field study.
A full description of the analytical method should be included with the results of the method validation study.

CONTINUING QUALITY CONTROL

Reagent or Solvent Blanks _____

Reagent or Solvent Spikes _____

Blank-Matrix Spikes _____

      Matrix _____ Spike Level _____

      Matrix _____ Spike Level _____

      Matrix _____ Spike Level _____

      Matrix _____ Spike Level _____

Actual Matrix Spikes _____

Replicate Matrix Analyses _____

Replicate Extract Injections _____

Confirmation Analyses _____

_____

_____

_____

ɹr Well Samples:

Primary Samples _____

Backup Samples _____

Field Blank Samples _____

Storage Dissipation Study _____

_____

_____

_____

_____

_____

_____

_____

_____

_____

Appendix C: Analytical Laboratory Specifications and Method Development Results

This appendix contains: analytical laboratory specifications, storage dissipation study results, method detection study results, and method validation study results.

In determining storage dissipation, influent water from CCCSD was split into 40 aliquots, each of which were spiked with 10 µg/L of both diazinon and chlorpyrifos. Ten samples were untreated, 10 were adjusted to pH 4, 10 were adjusted to pH 10, and 10 were added with 20 ml of methylene chloride. At the 24, 48, 72, and 120-hour intervals samples were extracted and analyzed. Storage dissipation study results were used to determine the optimum storage conditions prior to extraction.

In determining the method detection limit (MDL), blank matrix water was split into 7 aliquots which were identically spiked to the storage dissipation samples. The MDL is the product of the Student's t-value for the 99% confidence interval and the standard deviation. The resulting reporting limit is then set at 1-5 times the MDL depending on the matrix and instrument.

In the method validation phase, three replicates were analyzed at five spike levels (0.25, 0.5, 2.5, 10, and 20 µg/L). Control limits were developed based on the mean and standard deviations of the recovery results. The Upper Control Limit (UCL) is equivalent to the mean recovery plus twice the standard deviation. Likewise, the Lower Control Limit (LCL) is equivalent to the mean recovery minus twice the standard deviation.

With the exception of storage dissipation samples, all matrix water used during method development were filtered through a 1-µm pore size glass filter to remove larger particulates that were interfering with recovery of spiked analytes. Filtration was an important treatment for spiked samples because it reduced the loss of analytes from solution to solids in the matrix. Once bound to the solids, the analytes may not be removable during extraction. After filtration, the recovery results better reflect any deviation in the analytical procedure. All of the matrix water used for spiking had no detectable levels of diazinon and chlorpyrifos.

For both diazinon and chlorpyrifos, recovery generally decreases as spiked concentration increases. This phenomenon will be further discussed in Appendix D.

Appendix C

CALIFORNIA DEPARTMENT OF PESTICIDE REGULATION
ENVIRONMENTAL HAZARDS ASSESSMENT PROGRAM
ANALYTICAL LABORATORY SPECIFICATIONS

Proje Diazinon & Chlorpyrifos in Contra Costa Sewer Water		Lab:	APPL
Lab Project Director:	Diane Anderson	Phone:	(209) 275-2175
Project Chemist:	Paula Young	Phone:	"
QA Officer:	Pam Cooper	Phone:	"
EHAP Project Director:	Nan Singhasemanon	Phone:	(916) 324-4122
EHAP Lab Liason:	Cindy Garretson	Phone:	(209) 278-4198

Type of Analysis:

	Sample Type	Analysis For	Reporting Limit	Number of Samples
1	Matrix H2O for mdl study	diazinon and chlorpyrifos	0.05ug/l or better	8
2	Matrix H2O for Degradation	"	0.05ug/l or better	40
3	Matrix H2O for M.V.	"	0.05ug/l or better	15
4	Main Study Water Samples	"	0.05ug/l or better	166

Method Validation:	See Method Validation attachment
Degradation Study:	See Degradation Study attachment
Sample Storage:	Refrigerate H2O's (4°C)
Sample Extraction:	Extract within 7 days of receipt.
Analytical Standard Source:	In House Analytical Standards
Instrumentation:	GC/NPD
Continuing QC:	see Continuing Quality Control attachment
Extract Disposition:	Hold until advised by CDPR
Reporting	see Reporting attachment

Turnaround	3 weeks

Other Specifications:

MDL study must be run first. After an mdl is established and it is
determined whether or not there are any background levels or matrix interferences present then
spike levels can be set for the method validation and degradation studies.

Study to be performed in accordance with CDPR SOP Number QAQC001.00

Approved By: Cindy Garretson   Approved By: Date:

CDPR Representative   Lab Representative

| Please Read, Sign, and Return |

Pro   Director: Project Chemist:

CALIFORNIA DEPARTMENT OF PESTICIDE REGULATION
ENVIRONMENTAL HAZARDS ASSESSMENT PROGRAM
ANALYTICAL LABORATORY SPECIFICATIONS

METHOD DETECTION AND REPORTING LIMIT

METHOD DETECTION LIMIT

Determine a Method Detection Limit (MDL) according to the USEPA definition (40CFR, Part 136, B).

MATRIX	ANALYTE SPIKED	SPIKE LEVEL	REPS
CDPR Matrix H2O	diazinon and chlorpyrifos*	ug/l	7
CDPR Matrix H2O	NONE	NONE	1

Spiking Procedure: Using matrix provided by CDPR Prepare a total of 7 spiked samples per matrix.
    Analyze 1 Blank Matrix Sample per matrix.

* Refers to APPL In-House Analytical Standards

Documentation Requirements:
Each laboratory shall determine a method detection limit (mdl) and
a reporting limit (rl) for each analyte. Each laboratory shall also document their terms, definitions
and procedures for determing MDL and RL in their approved analytical method. Each laboratory
sha   ovide a copy of their approved analytical method before analyzing field samples. The results
from the method validation study will be used to establish recovery control limits for the field study.

REPORTING LIMIT

The Reporting Limit (RL) is 1 - 5 times the MDL depending on the analytical method and matrix.

Please Read, Sign, and Return

Project Director: Project Chemist:

CALIFORNIA DEPARTMENT OF PESTICIDE REGULATION
ENVIRONMENTAL HAZARDS ASSESSMENT PROGRAM
ANALYTICAL LABORATORY SPECIFICATIONS

DEGRADATION STUDY

MATRIX	ANALYTE SPIKED	SPIKE LEVEL	REPS
1) CDPR Matrix H2O	diazinon and chlorpyrifos*	10ug/l	10
2) CDPR Matrix H2O (pH 4)	diazinon and chlorpyrifos*	10ug/l	10
3) CDPR Matrix H2O (pH 8)	diazinon and chlorpyrifos*	10ug/l	10
4) CDPR Matrix H2O (solvent)	diazinon and chlorpyrifos*	10ug/l	10

* Refers to APPL In-House Analytical Standards
** To be determined by MDL Study

Spiking Procedure:

Using matrix provided by CDPR Prepare 10 reps per treatment spiked with 10ug/l each of diazinon
and chlorpyrifos. Keep samples refrigerated.

Treatments:
1) no treatment, measure and record matrix pH.
2) adjust pH to 4
3) adjust pH to 10
4) pour matrix into each of 10 1liter amber jars. Add   mls of

Analysis Procedure:

Pull 3 reps of each treatment at the specified sampling intervals and analyze.

Sampling Intervals:

Sampling Interval	Water Samples
Immediately Post Spiking	8
Approximately 12 HOURS	8
Approximately 48 HOURS	8
Approximately 96 HOURS	8
1 WEEK	8
Total	40

	Please Read, Sign, and Return	
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Project Director:		Project Chemist:

CALIFORNIA DEPARTMENT OF PESTICIDE REGULATION
ENVIRONMENTAL HAZARDS ASSESSMENT PROGRAM
ANALYTICAL LABORATORY SPECIFICATIONS

METHOD VALIDATION STUDY

METHOD VALIDATION

Run a Method Validation Study to establish Control Limits for these analytes in these matrices.
These Control Limits will be adhered to through the course of the study.

MATRIX	ANALYTE SPIKED	SPIKE LEVEL	REPS
CDPR Matrix H2O	diazinon and chlorpyrifos*	0.25ug/l	3
"	"	0.5ug/l	3
"	"	2.5ug/l	3
"	"	10ug/l	3
"	"	20ug/l	3
"	NONE	NONE	1

Spike Procedure: Using matrix provided by CDPR Prepare a total of 15 spiked samples per matrix.
      Analyze 1 Blank Matrix Sample per matrix.

* Refers to APPL In-House Analytical Standards

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CALIFORNIA DEPARTMENT OF PESTICIDE REGULATION
ENVIRONMENTAL HAZARDS ASSESSMENT PROGRAM
ANALYTICAL LABORATORY SPECIFICATIONS

CONTINUING QUALITY CONTROL

Matrix Blanks: *One Matrix Blank per matrix per extraction set. Matrices provided by CDPR*

(to test for laboratory contamination; glassware, matrix, instruments,etc.)

Blank Matrix Spikes: *One Pair Of Matrix Spikes Per Matrix Per Extraction Set*

(to evaluate method accuracy and precision based on previously established control limits)

MATRIX	ANALYTE SPIKED	SPIKE LEVEL	UNITS
CDPR Matrix H2O	diazinon and chlorpyrifos*		ug/l

Spiking Procedure: Add Analytical Standard at appropriate spike level to matrices provided by CDPR.

* *Refers to APPL In-House analytical standards*

QC Reporting Requirements:

1) Extraction Date.
2) Extraction set reference (APPL lab #s).
3) Report Date.
4) Method SOP#.
5) Spike matrix.
6) Analyte spiked.
7) Amount spiked.
8) Method Detection Limit.
9) Reporting Units.
10) Results.
11) % Recovery.
12) RPD. LIMIT = 30%
13) Previously established Control Limits:

Type of Analysis	General RPD Limits
LC/MS and NPD	30%
Inorganics	25%
VOA's, GC/MS, ECD, FID	20%

Analyte and Matrix	Lower Control Limit Mean - 2 x SD	Upper Control Limit Mean + 2 x SD
Diazinon in CDPR Matrix H2O		
Chlorpyrifos in CDPR Matrix H2O		

To be established

In Case of Failing QC:

If the Matrix Blank is positive or if the Duplicate Matrix Spike recoveries or
RPD's fall outside the previously established limits for this study APPL should immediately
notify the CDPR lab liason and cease sample analysis until; 1) the samples in the
failing extraction set have been rerun and meet QC standards or 2) the CDPR lab liason
and project leader agree to an alternative.

	Please Read, Sign, and Return	
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CALIFORNIA DEPARTMENT OF PESTICIDE REGULATION
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ANALYTICAL LABORATORY SPECIFICATIONS

REPORTING PROCEDURES

Completing the Chain of Custody Record:

1) Sign and date the box marked "Received for Lab by:".
2) Write in the Lab I.D. number in the appropriate space.
3) For those samples which contain no detectable amount write "none detected" and
    indicate the detection limit.
4) The chemist who analyzed the sample should sign and date in the appropriate space.
5) Write the date of extraction and analysis in the appropriate space.

Additional Specifications:

Matrix:	Report As:
1) Water	ug/l

Please Read, Sign, and Return

Project Director:

Project Chemist:

Table C1: Percent Recovery under Various Treatments from APPL's Diazinon Storage Dissipation Study in a Sewage Matrix *

Treatment	0 hr.	24 hrs.	48 hrs.	72 hrs.	120 hrs.
Untreated	74	54	52	52	44
Untreated	51	46	52	60	56
pH 4	44	11	0	0	0
pH 4	33	14	0	21	0
pH 10	12	10	47	47	61
pH 10	27	16	46	26	60
MeCl**	15	15	14	0.8	13
MeCl**	17	12	16	13	15

*Spike level = 10 µg/L, matrix = raw CCCSD influent, stored at 4°C, reporting limit = 0.05 µg/L

**20 ml of Methylene Chloride added.

Table C1: Percent Recovery under Various Treatments from APPL's Chlorpyrifos Storage Dissipation Study in a Sewage Matrix*

Treatment	0 hr.	24 hrs.	48 hrs.	72 hrs.	120 hrs.
Untreated	70	53	50	50	44
Untreated	22	47	53	56	53
pH 4	57	62	64	66	57
pH 4	65	55	56	43	53
pH 10	16	26	20	47	32
pH 10	29	27	19	30	31
MeCl**	20	19	20	10	19
MeCl**	21	17	20	21	20

*Spike level = 10 µg/L, matrix = raw CCCSD influent, stored at 4°C, reporting limit = 0.05 µg/L

**20 ml of Methylene Chloride added.

Appendix C

Table C3: APPL Method Detection Limit Study/Diazinon Results

Spike Level (µg/L)	Results (µg/L)	Percent Recovery	Mean	Standard Deviation
0.10*	0.108	108		
0.10*	0.083	83		
0.10*	0.096	96		
0.10*	0.104	104		
0.10*	0.096	96		
0.10*	0.114	114		
0.10*	0.102	102	100.4	10.0
0.10**	0.107	107		
0.10**	0.080	80		
0.10**	0.092	92		
0.10**	0.098	98		
0.10**	0.100	100		
0.10**	0.109	109		
0.10**	0.098	98	97.7	9.7

*HP-35 GC column used

**DB-5 GC column used

Appendix C

Table C4: APPL Method Detection Limit Study/Chlorpyrifos Results

Spike Level (µg/L)	Results (µg/L)	Percent Recovery	Mean	Standard Deviation
0.10*	0.074	74		
0.10*	0.053	53		
0.10*	0.078	78		
0.10*	0.081	81		
0.10*	0.069	69		
0.10*	0.093	93		
0.10*	0.083	83	75.9	12.6
0.10**	0.089	89		
0.10**	0.063	63		
0.10**	0.079	79		
0.10**	0.086	86		
0.10**	0.083	83		
0.10**	0.086	86		
0.10**	0.078	78	80.6	8.7

*HP-35 GC column used

**DB-5 GC column used

Table C5: APPL Method Validation Study Results for Diazinon*

Spike Level (µg/L)	Results (µg/L)	Percent Recovery	Mean	Standard Deviation	Coefficient of Variance	Lower Control Limit	Upper Control Limit
0.25	0.28	112					
0.25	0.26	104					
0.25	0.35	140	118.7	18.9	15.9	80.9	156
0.5	0.51	102					
0.5	0.55	110					
0.5	0.48	96	102.7	7.0	6.8	72.9	151
2.5	2.2	88					
2.5	2.3	92					
2.5	1.9	76	88.0	8.3	9.5	70.7	105
10	6.3	63					
10	4.5	45					
10	5.6	56	54.7	9.1	16.6	34.6	113
20	13	65					
20	13	65					
20	9.9	50	59.8	8.9	14.9	38.9	81.0
		Overall:	84.2	27.1	32.1	30.1	138

*Matrix = raw CCCSD influent, reporting limit = 0.05 µg/L

Table C6: APPL Method Validation Study Results for Chlorpyrifos*

Spike Level (µg/L)	Results (µg/L)	Percent Recovery	Mean	Standard Deviation	Coefficient of Variance	Lower Control Limit	Upper Control Limit
0.25	0.19	76					
0.25	0.18	72					
0.25	0.21	84	77.3	6.11	7.90	65.1	89.6
0.5	0.38	76					
0.5	0.42	84					
0.5	0.38	76	78.7	4.62	5.87	70.8	89.2
2.5	1.9	76					
2.5	2.0	80					
2.5	1.6	64	73.3	8.33	11.4	60.1	87.9
10	5.9	59					
10	4.2	42					
10	5.0	50	50.3	8.5	16.9	35.0	92.7
20	12	60					
20	11	55					
20	9.4	47	53.9	6.6	12.2	38.1	69.6
		Overall:	66.7	13.8	20.7	39.1	94.4

*Matrix = raw CCCSD influent, reporting limit = 0.05 µg/L

Appendix C

Appendix D: Laboratory Quality Control Results and Discussion

Overall, the 176 samples were analyzed in 21 extraction sets. An extraction set contained as few as one sample and as many as 18. Matrix spike (MS) recoveries ranged from 62.9% to 121.5% with an average of 79.5% and a standard deviation of 17.7% for diazinon (see Table D1 at the end of this appendix). For chlorpyrifos, the MS recoveries ranged from 51.5% to 96.6% with an average of 67.7% and a standard deviation of 13.8% (Table D2). The method validation performed at the beginning of the study established control limits of 30.1% to 138.0% for diazinon and 39.1% to 94.4% for chlorpyrifos. During the course of the study, two chlorpyrifos samples exceeded the upper control limit in batches which were extracted on 8/15/96 and 9/3/96 (95.5% and 96.6%, respectively). Diazinon MS recoveries did not exceed the control limits.

A C18 solid-phase extraction disk (trade name Empore™ developed by 3M) was used to extract analytes from the sample medium. The initial set of disks provided recoveries of 72.2% and 61.9% for diazinon and chlorpyrifos, respectively. APPL attempted to improve recovery by replacing this initial Empore™ disk with a larger diameter disk. The new extraction disk was employed during a period when most of the samples taken were commercial samples, and associated field and equipment blanks.

Recovery results were improved with the larger Empore™ disk as can be seen in the MS results. The disks were used on samples extracted on 8/15, 8/22, 8/28, and 9/3/96. The average MS recovery for these four extraction sets were 110.8% and 92.3% for diazinon and chlorpyrifos, respectively. Due to this change in sample extraction, the chlorpyrifos results from 8/15/96 and 9/3/96 did not technically exceed the 94.4% chlorpyrifos upper control limit which was established using the initial sample extraction and analysis method.

Early in the study, it was apparent that matrix effects did not have a major role in the low recoveries of either analytes. This was because recoveries of deionized water spikes (D.I. spikes) were close to or only marginally higher than the recoveries from MS spikes. If interference from the sewage matrix was pronounced, then the D.I. spike recoveries should have been significantly higher than the MS spike recoveries. Moreover, when the old disks were replaced, the recoveries of the D.I. spikes increased 16% for diazinon and 20% for chlorpyrifos. Assuming that the matrix and other factors

remained constant, some phenomena associated with the original set of Empore™ extraction disks were likely the main cause(s) of the lower recoveries.

Similar recovery problems experienced by several laboratories employing the Empore™ extraction disks prompted the manufacturer (3M) to conduct a follow-up investigation. Recent information released by 3M confirmed that low recoveries (40 - 70 percent) resulted when methylene chloride and other solvents with low water solubility were used to elute the disks (3M, 1997). 3M theorized that the methylene chloride is unable to completely displace the water that saturates the internal pores of the disks. Therefore, analyte molecules adsorbed in these regions are not efficiently displaced during the elution stage. 3M has since developed a modified procedure which will increase recovery when using methylene chloride as an eluting solvent.

This behavior seems to also explain the observation during method validation in which recovery decreased with increasing spike concentration. At lower spike concentrations (e.g. 0.25 and 0.5 µg/L), analytes may only have saturated perimeter surfaces of the disk. Subsequent elution with the extraction solvent were able to removed most of the analytes. The average percent recovery for diazinon and chlorpyrifos at these spike levels were 110% and 78%, respectively. At higher spike levels (e.g. 10 and 20 µg/L), the disks were more saturated with analytes. Recall that methylene chloride is inefficient at eluting the internal pores of the disks. Thus, the average percent recovery for diazinon and chlorpyrifos at these spike levels were 57% and 52%, respectively.

The low recoveries of the initial disks and their subsequent replacement during the study prompted the adjustment of the all the analytical results based on the batch's average matrix spike recovery. For example, if the raw result for a sample was 60 ng/L, and the average matrix spike recovery for that batch of samples was 50%, the recovery-adjusted concentration would be 120 ng/L. Raw data was adjusted up by as much as 160% for diazinon, and 190% for chlorpyrifos. Conversely, raw data from four diazinon batches had to be adjusted down since the replacement disks provided diazinon recoveries that were higher than 100%. For the batch with the highest diazinon recovery, the raw data was reduced to 82% of their reported values. The adjustment of the data to 100% recovery was necessary to allow more consistent comparisons and calculations among the entire data set.

Appendix D

Reporting limits were also recovery-adjusted based on the batch matrix spike recoveries. Thus, after adjustment, the reporting limit is no longer fixed to the default value of 50 ng/L. The revised reporting limits are now denoted by a "less than" symbol followed by a numeric value (i.e. <46 or <65). Adjusted reporting limits are included in Tables D1 and D2.

The relative percent difference (RPD) is the difference between duplicate MS measurements divided by the average of the two measurements multiplied by 100. This result gives a general assessment of the precision of the method for that extraction set. For the GC/NPD method, an RPD limit of 30% was established by APPL. The average RPD of diazinon and chlorpyrifos were 11.7% and 10.3%, respectively. None of the extraction sets exceeded the RPD limit.

In addition to the batch QC samples, 8 in-house blind matrix spikes were analyzed by APPL (Table D3). Blind spikes were submitted in pairs in four extraction sets. The holding time between spiking and extraction were 6, 14, 6, and 8 days for sample pairs 300/301, 312/313, 310/311, and 306/307, respectively. The eight samples were filtered CCCSD influent samples spiked at the 250 ng/L for both analytes. Of these eight blind spikes, sample pair 306/307 belongs in a sample batch that used the new Empore™ disks. These two blind spikes have an average of 255 ng/L (102% recovery) for both diazinon and chlorpyrifos. The six other blind spikes have an average of 162 ng/L (65% recovery) for diazinon and 155 ng/L (62% recovery) for chlorpyrifos.

Two split extract samples were also sent from APPL to CDFA laboratory in Sacramento for comparative analysis (Table D4). APPL's analysis of the first CCCSD influent sample-extract quantified 140 ng/L for diazinon and <50 ng/L for chlorpyrifos. CDFA analysis of the same extract resulted in 169 and <50 ng/L, in the same order. The RPD of the diazinon results was 18.7%. For the second extract split, APPL's results were 8,900 and 2,200 ng/L for diazinon and chlorpyrifos. CDFA's analysis of the same extract found 4,350 and 1680 ng/L in the same order. The RPDs of this extract split were 68.7% for diazinon and 27.8% for chlorpyrifos. A review of sample handling procedures for the second extract pair showed that they were analyzed 2 weeks apart. The first extract pair was only analyzed 2 days apart.

Appendix D

Laboratory Quality Control Results

Appendix D

	Extraction Date	Table D1: Diazinon Results					
Sample Numbers	Extraction Date	Average Percent Recovery*	Percent Recovery Matrix Spike #1*	Percent Recovery Matrix Spike #2*	Relative Percent Difference	Percent Recovery Laboratory Control Sample**	Adjusted Reporting Limit (ng/L)
1,5,9,14,18,22,26,30,300, 301	6/25/96	64.6	71.8	57.4	22.3	66.2	<77
30	6/29/96	76.5	76.4	76.6	0.3	78.8	<65
31,32,33	7/6/96	98.0	88.0	108.0	20.4	75.6	<51
34,35,36,37,38,42,43,44,45,4 6,47,48,49,50,51,52,53	7/13/96	68.5	71.2	65.8	7.9	78.6	<73
54,55,56,57,58,59,60,61, 62,63,64,65,66,	7/18/96	72.6	66.4	78.8	17.1	66.6	<69
67,68,69,70,71,72,78,79,80,8 1,82,83,84,85,86,87,88,90	7/20/96	74.3	78.0	70.6	10.0	73.6	<67
89,91	7/23/96	78.4	74.0	82.8	11.2	70.2	<64
92,93,99	7/25/96	76.5	86.2	66.8	25.4	88.8	<65
94,101,105,111,115	7/27/96	79.3	78.6	80.0	1.8	71.2	<63
119,124,130,194,205,214, 199,200,216	7/31/96	65.2	69.0	61.4	11.7	83.2	<77
145,201,202,203,204	8/5/96	80.0	77.8	82.2	5.5	74.0	<63
147,157,133,134,135,136, 172,173,163,167	8/9/96	62.9	67.0	58.8	13.0	62.6	<79
312,313,137,138,139,140, 174,175	8/13/96	62.9	67.0	58.8	13.0	65.5	<79
176,177,178,179,228,258, 220,227,226,219,234,141, 142,143,144	8/15/96	104.0	98.0	110.0	11.5	110.0	<48
169,148,149,171,310,311	8/16/96	64.1	68.0	60.2	12.2	58.0	<78
235,236,237,238,239,240, 241,242,243,244,245,246, 247,248,181,182,183,184	8/17/96	72.1	67.6	76.6	12.5	53.4	<69
249,250,251,252,265,266, 267,268	8/22/96	108.0	113.0	103.0	9.3	73.4	<46
269,270,271,272,273,274, 306,307	8/28/96	109.5	110.2	108.8	1.3	85.6	<46
185,186,275	9/3/96	121.5	113.0	130.0	14.0	79.0	<41
187,188,190,192,276,277	9/7/96	65.4	70.4	60.4	15.3	52.2	<76
278,279,280,281,282,283, 284	9/13/96	65.6	67.4	63.8	5.5	71.2	<76
Average		79.5			11.5	73.2	
Standard Deviation		17.7			6.6	12.8	
Coefficient of Variance		4.5			1.7	5.7	

* Matrix spikes done at 250 ng/L in sewage.
** Laboratory control samples done at 250 ng/L in deionized water

Table D2: Chlorpyrifos Results

Sample Numbers	Extraction Date	Average Percent Recovery*	Percent Recovery Matrix Spike #1*	Percent Recovery Matrix Spike #2*	Relative Percent Difference	Percent Recovery Laboratory Control Sample**	Adjusted Reporting Limit (ng/L)
1,5,9,14,18,22,26,30,300, 301	6/25/96	67.8	71.8	63.8	11.8	69.6	<74
30	6/29/96	67.8	67.0	68.6	2.4	71.0	<74
31,32,33	7/6/96	77.2	69.0	85.4	21.2	64.2	<65
34,35,36,37,38,42,43,44,45,4 6,47,48,49,50,51,52,53	7/13/96	65.7	65.2	66.2	1.5	84.6	<76
54,55,56,57,58,59,60,61, 62,63,64,65,66,	7/18/96	61.7	55.2	68.2	21.1	68.2	<81
67,68,69,70,71,72,78,79,80,8 1,82,83,84,85,86,87,88,90	7/20/96	59.5	61.8	57.2	7.7	69.6	<84
89,91	7/23/96	61.1	57.2	65.0	12.8	77.8	<82
92,93,99	7/25/96	55.7	63.6	47.8	28.4	91.0	<90
94,101,105,111,115	7/27/96	56.5	55.2	57.8	4.6	75.4	<89
119,124,130,194,205,214, 199,200,216	7/31/96	60.6	66.4	54.8	19.1	81.4	<83
145,201,202,203,204	8/5/96	70.4	69.8	71.0	1.7	69.4	<71
147,157,133,134,135,136, 172,173,163,167	8/9/96	51.5	54.4	48.6	11.3	56.2	<97
312,313,137,138,139,140, 174,175	8/13/96	51.5	54.4	48.6	11.3	69.0	<97
176,177,178,179,228,258, 220,227,226,219,234,141, 142,143,144	8/15/96	95.5	91.0	100.0	9.4	111.0	<52
169,148,149,171,310,311	8/16/96	61.5	64.2	58.8	8.8	61.8	<81
235,236,237,238,239,240, 241,242,243,244,245,246, 247,248,181,182,183,184	8/17/96	68.4	66.8	70.0	4.7	55.2	<73
249,250,251,252,265,266, 267,268	8/22/96	88.4	92.0	84.8	8.1	80.2	<57
269,270,271,272,273,274, 306,307	8/28/96	88.8	88.2	89.4	1.4	93.4	<56
185,186,275	9/3/96	96.6	95.2	98.0	2.9	87.0	<52
187,188,190,192,276,277	9/7/96	56.8	61.4	52.2	16.2	49.8	<88
278,279,280,281,282,283, 284	9/13/96	58.6	59.4	57.8	2.7	73.0	<85
Average		67.7			9.9	74.2	
Standard Deviation		13.8			7.6	14.2	
Coefficient of Variance		4.9			1.3	5.2	

* Matrix spikes done at 250 ng/L in filtered sewage matrix using a 1-micron pore size filter paper.
** Laboratory control samples done at 250 ng/L in deionized water.

Table D3: Blind Spike Results

Sample Numbers	Date Spiked	Date Extracted	Diazinon* (ng/L)	Chlorpyrifos* (ng/L)	Diazinon Recovery (%)	Chlorpyrifos Recovery (%)
300	6/19/96	6/25/96	160	160	64	64
301	6/19/96	6/25/96	200	200	80	80
312	7/30/96	8/13/96	140	140	56	56
313	7/30/96	8/13/96	160	150	64	60
310	8/9/96	8/16/96	180	150	72	60
311	8/9/96	8/16/96	130	130	52	52
306**	8/20/96	8/28/96	240	240	96	96
307**	8/20/96	8/28/96	270	270	108	108

* Samples were spiked with 250 ng/L of analyte in a filtered sewage matrix using a 1-micron pore size filter paper. The reporting limit was 50 ng/L.

** New extraction disks used

Table D4: Split Sample Extracts

Extract Pair Number	Diazinon (ng/L)	Chlorpyrifos (ng/L)	RPD (%)	RPD (%)
1 (APPL)	140	<50	18.7	n/a
1 (CDFA)	169	<50	18.7	n/a
2 (APPL)	8900	2200	68.7	27.8
2 (CDFA)	4350	1680	68.7	27.8

Appendix E: Split Sample Comparison

Sewage from various source types (residential, commercial, influent, etc.) was split and sent to several participating laboratories for comparative analysis. The exercise was conducted to see if similar results could be attained in analyzing for diazinon and chlorpyrifos in a complex matrix. Two rounds of split sample comparisons were completed during the course of the study. The first began in mid-June and comprised of seven samples. APPL, CCCSD, Aqua-Science, and Dow-Elanco analytical laboratories analyzed samples in this first round. The second round began in the end of July and continued into August for a total of 19 samples. In addition to the participating laboratories in the previous round, the Ciba-Geigy laboratory participated in the second round. The sample extraction and analytical method of each laboratory is included at the end of this Appendix.

Samples to be split were taken in a clean 9.5-L glass composite jar and immediately taken to the CCCSD cargo bay. Samples were swirled vigorously for 30 seconds after which a 500-ml aliquot was poured through a clean glass funnel into the first 1-liter amber glass bottles. Swirling continued for another 10 seconds after which another 500-ml aliquot was decanted into the subsequent bottle. This process continued until the last bottle of the split was reached. At this point, the bottles were filled in the reverse order. The swirling time prior to each fill was increased to 15 seconds. Samples were then refrigerated until they were ready to be delivered or shipped out to the participating laboratories with packing materials and blue ice. During the second round, attempts were made to synchronize the extraction dates to minimize deviations due to differing holding times.

Particulate-filled and heterogeneous sewage medium produce splits that vary in the amount and type (bound versus dissolved) of analytes present. This variability cannot be measured based on the original design of this split sample analysis. Furthermore, the transport time and storage condition for each split depend on its destination. For example, split samples bound for AQUA-Science and CCCSD were delivered on the same day they were processed at CCCSD's Bay 11. Thus, these samples were immediately refrigerated and had better temperature control. Samples bound for APPL, Ciba-Geigy, and Dow-Elanco were shipped overnight, and arrived at these laboratories at least a day later. Improvements were made in the second round by thoroughly cooling down all the samples overnight prior to transport.

<div align="center">Appendix E</div>

Differences between holding times of each split created additional variability. The holding time is defined here as the time period from when the sample was taken until it was extracted (or analyzed in the case of ELISA). Holding times differed depending on when each laboratory was able to process its sample. During the first round, holding times were more variable. For the second round, however, efforts were made to shorten and synchronize the holding times to improve comparability of the results. The raw split sample analytical results and sample holding times are presented in Table E1 (Diazinon) and in Table E2 (Chlorpyrifos).

Table E1: Split Sample Diazinon Concentrations (ng/L) and Days Held (in parenthesis)

APPL Sample Number	Sample Source*	Sample Type**	APPL Results	CCCSD Results	Aqua-Science Results	Dow-Elanco Results	Ciba-Geigy Results
1	Bay 11	EB	<50 (6)	<10 (5)	<30 (1)	<25 (20)	
5	I01	FB	<50 (6)	<10 (5)	<30 (1)	<25 (20)	
9	I01	G	140 (6)	87 (5)	333 (1)	248 (20)	
14	C11	G	8700 (5)	6270 (6)	31500 (1)	14270 (19)	
18	R05	G	50 (5)	16 (6)	35 (1)	53 (19)	
22	C12	G	<50 (5)	10 (5)	<30 (1)	<25 (19)	
26	I01	C	610 (7)	383 (5)	1700 (4)	1100 (18)	
94	I01	C	210 (7)	235 (n/a)		234 (14)	58 (11)
101	Bay 11	RB	<50 (5)	<10 (n/a)	<30 (4)	<25 (12)	14 (9)
105	C16	FB	<50 (5)	<10 (n/a)	<30 (4)	<25 (12)	<10 (9)
111	I01	C	190 (4)	61 (n/a)	218 (3)	197 (11)	120 (8)
115	C16	C	910 (4)	927 (n/a)	829 (3)		
119	C16	C	410 (7)	107 (n/a)	280 (6)	461 (12)	420 (7)
130	C11	FB	<50 (6)	<10 (n/a)	<30 (6)	<25 (12)	<10 (6)
124	Bay 11	RB	<50 (6)	<10 (n/a)	<30 (6)	<25 (12)	<10 (6)
205	C11	C	8900 (6)	8383 (n/a)	18060 (7)	15850 (12)	6600 (6)
214	C11	C	8700 (5)	8125 (n/a)	20200 (6)	13660 (11)	6200 (5)
194	C11	C	13000 (4)	9336 (n/a)	20280 (5)	14120 (10)	5500 (4)
157	I01	C	120 (4)	59 (n/a)	160 (4)	153 (3)	120 (4)
147	I01	FB	<50 (4)	<10 (n/a)	<30 (4)	<25 (3)	<10 (4)
163	I01	C	470 (3)	466 (n/a)	752 (3)	966 (4)	950 (3)
167	I01	C	330 (2)	390 (n/a)	533 (2)	689 (3)	560 (2)
220	I01	C	130 (7)		224 (5)	139 (3)	210 (7)
228	I01	C	160 (6)		168 (4)	150 (5)	250 (6)
258	I01	C	190 (5)		316 (3)	266 (7)	420 (5)
234	I01	C	220 (4)		365 (2)	336 (5)	

* Bay 11 = CCCSD field operations facility, I01 = CCCSD influent, C# = commercial site, R# = residential site

** EB = equipment blank, FB = field blank, RB = rinse blank, G = grab, C = composite, n/a = data not available

First round split results are shaded.

Appendix E

Table E2: Split Sample Chlorpyrifos Concentrations (ng/L) and Days Held (in parenthesis)

APPL Sample Number	Sample Source*	Sample Type**	APPL Results	CCCSD Results	Aqua-Science Results	Dow-Elanco Results	Ciba-Geigy Results
1	Bay 11	EB	<50 (6)	<10 (5)	<50 (1)	<25 (20)	
5	I01	FB	<50 (6)	<10 (5)	<50 (1)	<25 (20)	
9	I01	G	<50 (6)	28 (5)	111 (1)	62 (20)	
14	C11	G	2300 (5)	5940 (6)	3994 (1)	1290 (19)	
18	R05	G	50 (5)	31 (6)	154 (1)	65 (19)	
22	C12	G	<50 (5)	<10 (5)	58 (1)	<25 (19)	
26	I01	C	100 (7)	71 (5)	191 (4)	123 (18)	
94	I01	C	73 (7)		150 (4)	173 (14)	
101	Bay 11	RB	<50 (5)	<10 (n/a)	<50 (4)	<25 (12)	
105	C16	FB	<50 (5)	<10 (n/a)	<50 (4)	<25 (12)	
111	I01	C	110 (4)	81 (n/a)	253 (3)	210 (11)	
115	C16	C	200 (4)	265 (n/a)	531 (3)		
119	C16	C	190 (7)	302 (n/a)	266 (6)	877 (12)	
130	C11	FB	<50 (6)	<10 (n/a)	<50 (6)	<25 (12)	
124	Bay 11	RB	<50 (6)	<10 (n/a)	<50 (6)	<25 (12)	
205	C11	C	2200 (6)	4050 (n/a)	9821 (7)	8140 (12)	
214	C11	C	580 (5)	1407 (n/a)	2677 (6)	2080 (11)	
194	C11	C	620 (4)	1870 (n/a)	928 (5)	1440 (10)	
157	I01	C	120 (4)	85 (n/a)	269 (4)	222 (3)	
147	I01	FB	<50 (4)	<10 (n/a)	60 (4)	<25 (3)	
163	I01	C	110 (3)	120 (n/a)	351 (3)	258 (4)	
167	I01	C	94 (2)	151 (n/a)	263 (2)	209 (3)	
220	I01	C	200 (7)		140 (5)	101 (3)	
228	I01	C	180 (6)		139 (4)	172 (5)	
258	I01	C	130 (5)		175 (3)	213 (7)	
234	I01	C	180 (4)		338 (2)	418 (5)	

* Bay 11 = CCCSD field operations facility, I01 = CCCSD influent, C# = commercial site, R# = residential site

** EB = equipment blank, FB = field blank, RB = rinse blank, G = grab, C = composite, n/a = data not available

First round split results are shaded.

In the first round, APPL, AQUA-Science, CCCSD, and Dow-Elanco laboratories each analyzed a total of seven samples. These samples consisted of one field blank, one equipment blank, one residential, two commercial, and two influent samples. The field and equipment blanks and a commercial sample were at or below the detection limit and were not used to calculate the relative ratio. However, it remained important to show that all the laboratories reported "no detection" or at levels close to their respective detection limits. The remaining four samples had concentrations at high enough levels for a relative comparison.

In the second round, the four original laboratories participated with the addition of Ciba-Geigy (note that Ciba-Geigy's laboratory only analyzed for diazinon). A total of 19 samples were split and analyzed. These samples included three field blank, two equipment blank, five commercial, and nine influent samples. A large number of commercial and influent samples were split because concentration levels were expected to be high enough for comparison. Five blanks were also sent to each laboratory for analysis.

The five commercial and nine influent samples contained high enough concentrations of diazinon and chlorpyrifos for comparison. CCCSD was unable to analyze five samples. Ciba-Geigy was also unable to analyze one sample due to bottle breakage. Four samples were deemed inadequately stored after arrival at the Dow-Elanco laboratory although the samples were later analyzed. These four samples were marked and were not used for comparison.

It was not practical to statistically determine the similarities and differences between the analytical methods of the laboratories involved. Differences in particulate contents, holding times, transport times, and transport conditions may have produced split samples that vary in diazinon and chlorpyrifos concentrations. Thus, differences in the results were not necessarily attributable to differences in each laboratory's analytical method alone. For this reason, statistical analyses that compare performances of analytical methods such as a linear regression between each laboratory's results may not be very meaningful. Recommendations to improve the comparability of results for future comparisons involving a raw sewage matrix are made at the end of this appendix. Subsequent discussion of split sample results are done in a more qualitative manner.

APPL's analytical results were compared to those of AQUA-Science, CCCSD, Ciba-Geigy, and Dow-Elanco. All data used for comparison in this appendix are raw and not recovery adjusted. The average ratios of APPL results to other laboratories for the first and second round of the split sample comparison are shown in Table E3.

Table E3: Average Ratios of Analytical Results by Rounds.

Laboratory	Average Ratio of Analytical Results (First Round)*		Average Ratio of Analytical Results (Second Round)**	
	Diazinon	Chlorpyrifos	Diazinon	Chlorpyrifos
APPL:APPL	1.00	1.00	1.00	1.00
AQUA-Science:APPL	2.37	2.24	1.38	2.28
CCCSD:APPL	0.57	1.30	0.76	1.59
Ciba-Geigy:APPL	n/a	n/a	1.16	n/a
Dow-Elanco:APPL	1.57	1.03	1.39	2.33

* First round ratios based on 4 diazinon and 3 chlorpyrifos results which were above the reporting limit.

** Second round ratios based on 14 diazinon and 14 chlorpyrifos results which were above the reporting limit.

The four samples in the first round were pooled with the 14 samples in the second round to produce a data set for the entire split sample comparison. The combined first and second round results ratios are shown in Table E4.

Table E4: Average Ratios of Analytical Results for the Entire Split Sample Comparison.

Laboratory	Average Ratio of Analytical results (First + Second Round)		Number of Comparable* Results (total = 18)	
	Diazinon	Chlorpyrifos	Diazinon	Chlorpyrifos
APPL:APPL	1.00	1.00	18	17
AQUA-Science:APPL	1.69	2.27	17	17
CCCSD:APPL	0.73	1.52	14	12
Ciba-Geigy:APPL	1.16	n/a	12	0
Dow-Elanco:APPL	1.43	2.09	17	16

* "Comparable" signifies sample results available that were above the reporting limit.

Average ratios were calculated to estimate the relative magnitude of a particular laboratory's results to that of APPL. APPL was selected as the reference laboratory because its data was used in this report. Average ratios which are less than 1.00 suggest that split sample results from a particular laboratory have a tendency to be lower than APPL, and vice versa. For example, the CCCSD:APPL diazinon ratio of 0.73 means that for the 14 common split samples between the two laboratories, CCCSD was producing on average 73% of the value produced by APPL. As another example, the AQUA-Science:APPL chlorpyrifos ratio of 2.27 means that for the 18 common split samples between the two laboratories, AQUA-Science was producing 227% of the value produced by APPL.

These ratios do not necessarily show that the results from a particular laboratory always followed this relationship. Plots of results ratios of APPL to other laboratories for individual split samples (Figures E1&E2 on the following page) illustrate this. As an example, although the AQUA-Science:APPL chlorpyrifos is 2.27, two of 17 split samples between the two laboratories show that AQUA-Science produced lower results than APPL (below the ratio of 1.00). Six of 17 results were between the ratios of 1.00 and 2.00. Five of 17 results were between the ratios of 2.00 and 3.00. The remaining four results were between the ratio of 3.00 and 5.00.

The coefficient of variance (CV) calculated for each ratio shows the degree of variation that exists between each pair of sample to the next. CV values in this case represent the standard deviation of each laboratory's results ratio divided by the mean of each laboratory's results ratio. Diazinon CV values for AQUA-Science, CCCSD, Ciba-Geigy, and Dow-Elanco to APPL ratios were 45, 42, 55, and 26%, in that order. Similarly, chlorpyrifos CV values for AQUA-Science, CCCSD, and Dow-Elanco were also high at 49, 53, and 54%, respectively.

The average ratios of analytical results shown in Table E4 indicate that APPL's diazinon and chlorpyrifos results were generally lower than those from other participating laboratories with the exception of CCCSD for diazinon. After comparing APPL's analytical methods and quality control data with those of the other laboratories, the difference in the matrix spike (MS) recoveries between the laboratories may explain some of the difference in the analytical results. The average diazinon MS recoveries for AQUA-Science, CCCSD, Ciba-Geigy, and Dow-Elanco were 104, 82, 97, and

Figure E1: Diazinon Results Ratio of Participating Laboratories to APPL
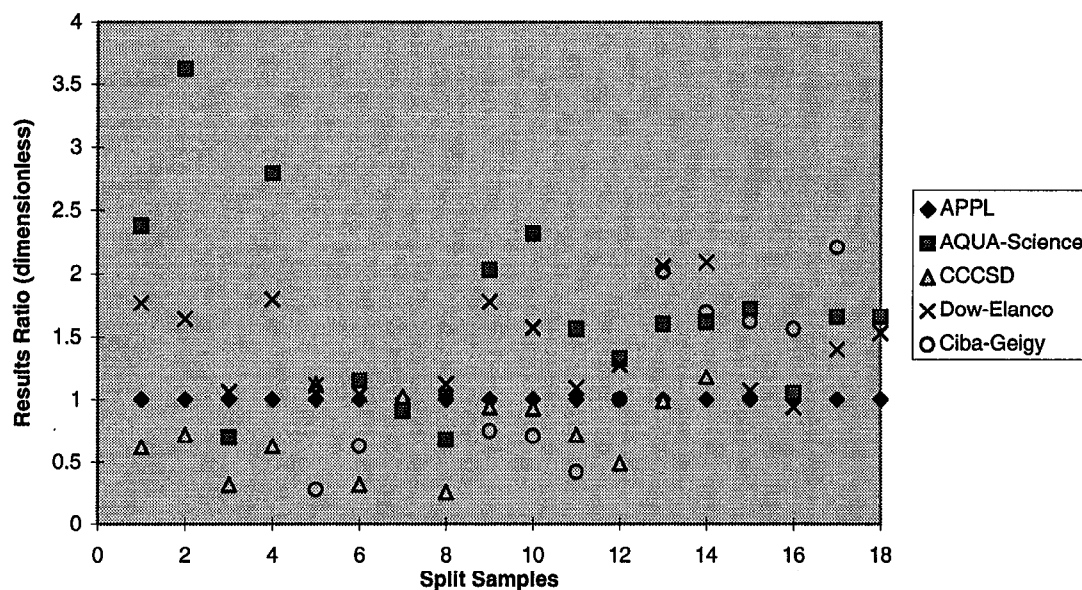
Figure E2: Chlorpyrifos Results Ratio of Participating Laboratories to APPL

Appendix E
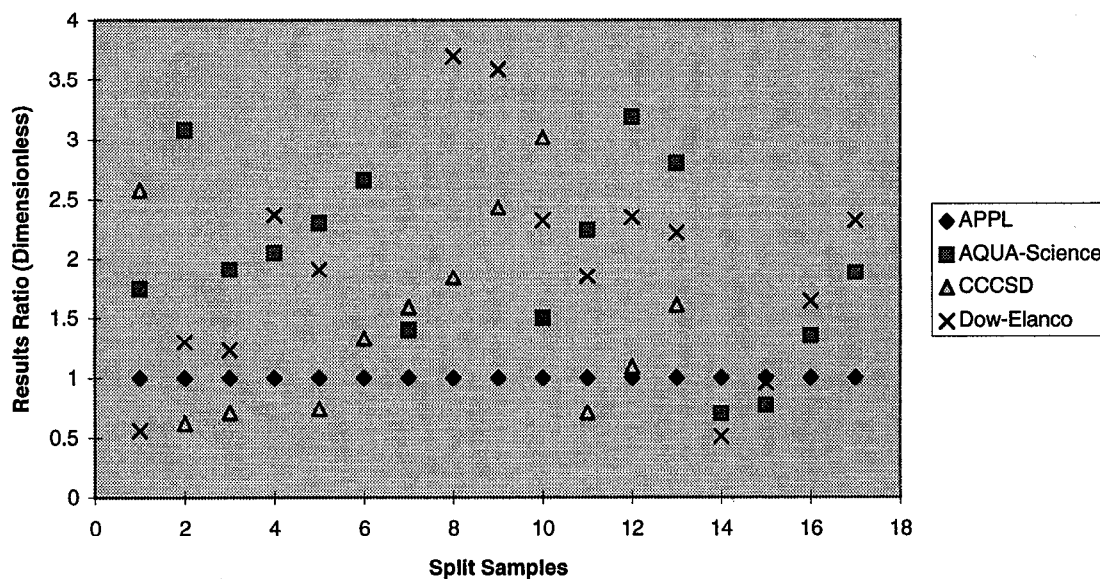
100%, respectively. The average chlorpyrifos MS recoveries for AQUA-Science, CCCSD, and Dow-Elanco were 109, 100, and 96%, respectively. For comparison, the average APPL MS recoveries for split sample batches were 75 and 66% for diazinon and chlorpyrifos, respectively. As previously discussed in Appendix D, the inefficiency of methylene chloride in eluting the original set of Empore™ extraction disks likely caused lower recoveries.

For a more accurate measurement of performance in future studies, several tasks are recommended. First, the degradation rates of diazinon and chlorpyrifos in raw sewage under various storage conditions need to be quantified. Second, individual spikes of organic-free water with carefully measured amounts of diazinon and chlorpyrifos should also be analyzed by each laboratory. Third, if splitting of raw sewage samples are going to be done, the variability of the splitting technique itself needs to be measured. This determination should focus on quantifying the amount of solids in each split. Furthermore, the improvement of temperature storage conditions prior to shipment as well as the minimization and synchronization of holding times will help keep splits more identical prior to analysis.

In addition to these recommendations, peer review of the extraction and analytical procedures of each laboratory is recommended particularly if the procedures used are new or experimental. A minimum level of quality control data needs to be established and shared in a timely manner. Statistical goals should also be established prior to the initiation of a comparison. Additional items will need to be considered based on specific requirements of future study objectives.

Central Contra Costa Sanitary District Analytical Method for Diazinon and Chlorpyrifos

STANDARD OPERATING PROCEDURE

Diazinon and Chlopyrifos (Dursban) by SIMM
(revised 1/1996)

1. SUMMARY:

A measured amount of sample is spiked with diazinon-d10 serving as internal standard and is extracted with methylene chloride using continuous liquid-liquid extraction technique at pH 2 for 18 hours. The extract is dried with anhydrous sodium sulfate and concentrated to a final volume of 1.0 ml and then analyzed using a GC/MS on selected ion monitoring acquisition mode. Quantitation is performed by internal standard method. THE ACQUIRED DATA PROVIDES A TWO DIMENSIONAL ANALYSIS BASED ON RETENTION TIME AND SPECTRAL IDENTIFICATION OF TWO ION PEAKS.

The following Standard Operating Procedures should be referred to during the analysis of sample for Diazinon and Dursban:

- Sample Bottle Preparation
- GC/MS QA/QC for SIMM
- Continuous Liquid-Liquid Extraction
- Gel Permeation Chromatography Sample Clean-up
- Alumina-Silica Gel Sample Clean-up
- GC/MS Setup and Preparation for Data Acquisition

1.1 List of Analytes:

order of elution:	CAS:	analyte	monitoring ion
1	n/a	Diazinon-d10 (IS)	314.16
2	333-41-5	Diazinon	304.10
2			137.07
3	2921-88-2	Chlorpyrifos	313.96
3			196.92

2. MATERIALS:

- For materials and equipment necessary to carry out liquid-liquid extraction steps, see liquid-liquid extraction S.O.P.
- Autosampler vials: Supelco cat 2-7323
- Vial inserts: Chromacol cat 03-MTV
- Base springs for vial inserts: Chromacol cat MTS-1
- Crimp vial caps: Chromacol cat 11-AC-ST101
- Fused silica capillary analytical column: DB-5ms 30 m, 0.32 mm, 0.25 um: J&W cat 123-5532

2.1 Reagent

- Diazinon-d10: from Cambridge Isotope Laboratories (800) 322-1174, cat. DLM. Prepare a 10 ug/ml secondary standard solution from this neat standard in methylene chloride.
- Diazinon: from Accustandard (800) 442-5290, cat. P-033S, 100 ug/ml in methanol. This is a primary standard solution.

- Chlorpyrifos (dursban): from Accustandard, cat. P-094S. 100 ug/ml in methanol; this is a primary standard solution.

3. QUALITY CONTROL, QUALITY ASSURANCE:

- Follow instruction in the QA/QC for SIMM S.O.P. Make sure to meet criteria for DFTPP, initial calibration, second source standard calibration check, and continuing calibration check before proceeding to sample injection.

4. SAMPLE COLLECTION and PRESERVATION:

- Use an amber glass bottle with Teflon-lined cap. Prepare the bottle following the "Sample Bottle Preparation" S.O.P.
- Collect 1000 ml sample in a cleaned bottle and refrigerate the bottle immediately if the sample is not extracted within next two hours.

5. SAMPLE EXTRACTION:

- See the "Liquid-Liquid Extraction" S.O.P.

6. SAMPLE CLEAN-UP:

- If necessary. See appropriate sample clean-up method S.O.P.

7. INSTRUMENT PREPARATION:

7.1 GC Temperature Program:

hold time (mins)	temp (C)	rate (C/min)
0.00		
1.00	55	15
0.00	150	5
0.00	200	35
5.00	280	

valves (mins)	
purge	split
1.0	1.0

Injector temp: 250 C
MS interface: 250 C

- Now follow instruction in the "GC/MS Setup and Preparation for Data Acquisition" S.O.P.

8. SAMPLE INJECTION and DATA ACQUISITION:

8.1 Sample List

- A basic sample list (in MassLab) should contain the information like the example followed:

bottle	datafile	text	type	conc	peakdetection
1	OPP0012	1000 ng/ml OPP std	standard	1000	no
2	OPP0013	Raw Infl lab # 3065	unknown	0	no

quantify	quantifymethod	calibrationfile	ASfile	GCfile	MSfile
no	OPPSIM	OPP1227	OPP	OPP	OPPSIM
no	OPPSIM	OPP1227	OPP	OPP	OPPSIM

- Enter appropriate information for:

    . bottle number,
    . datafile name,
    . sample description text,
    . type (standard or unknown),
    . concentration (non-zero for standard, zero for unknown),
    . peak detection (N to acquire data, Y to detect peaks),
    . quantify (N when acquire data and detect peaks, Y to quantify integrated peaks),
    . quantify method,
    . calibration file name,
    . autosampler method file name,
    . GC method file name,
    . acquisition method file name.

8.2 Autosampler Vial Preparation:

- Clearly label autosampler vials.
- Insert a bottom spring and a vial insert into the labeled vial.
- Transfer about 100 ul of sample extracts into vial insets and crimp cap the vials.
- Place vials on the autosampler's tray in their exact positions assigned on the sample list above.

8.3 System Verification and Start:

- Review over the "GC/MS Setup and Preparation for Data Acquisition" S.O.P. one more time to make sure that parameters entered are correct and everything is ready.
- Turn on the filament.
- From the sample list menu, select "acquire data" but **not** "measure data"
- Execute sample injection and acquisition by starting the sample list

9. DATA PROCESSING:

- See "Data Processing " s.o.p.

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STANDARD OPERATING PROCEDURE

Continuous Liquid-Liquid Extraction
(revised 1/1996)

</div>

1. MATERIAL:

1.1 Equipment:

- Continuous liquid-liquid extractor equipped with glass connecting joints and stopcorks requiring no lubrication.
- Heating water jacked 10 ml Kuderna-Danish concentrator tube with ground-glass stoppers.
- Vials: 1.8 ml amber glass with Teflon-lined screw cap
- Micro-pipetters capable of delivering 10.0 ul, 20.0 ul, 50.0 ul, 100.0 ul volumes

1.2 Reagents:

- Reagent water: UV treated OrganicPure Barnstead water
- Anhydrous sodium sulfate: Granular, fired in a muffler furnace at 550 C overnight
- Sulfuric acid 6N free of contaminations
- Acid surrogates mixture
- Base-Neutral surrogates mixture
- Base-Neutrals/Acids spiking mixture

2. PROCEDURE:

2.1 Recommended Sample Sizes:

Raw influent:	1000 ml
Final effluent:	1000 ml
Source control samples:	100 ml
STLC extracts:	20 ml for cake, 100 ml for others
TCLP extracts:	20 ml for cake, 100 ml for others
Septic tank samples:	10 ml

2.2 Procedure:

- Fill out a sample work-up sheet as the procedure is being carried through.

- Set out the Liquid/Liquid extraction pieces that will be needed for the number of samples you have. Rinse all glassware twice with methanol and once with methylene chloride.

- Assemble the extraction apparatus and turn on the water bath so it can reach 75 C. Open the valve on the water bath such that water now circulates into the water-jacketed Kuderna-Danish concentrator portion of the extraction apparatus.

- Measure out 450 ml of Methylene Chloride and pour into the main body of the Liquid/Liquid extractor.

LIQEXTR.WPD

- Add 1000 ml of sample or any other acceptable sample size depending on how dirty the sample is.

- Add to the sample about 3 g of sodium sulfate.

- Acidify the sample to pH 2 by adding 1 ml of 6N Sulfuric acid; Verify the pH using a pH paper strip.

- Add the following compounds to all samples prepared for 625 extraction:
        _ 250 ul of a 200 ug/ml Acid Surrogate Solution
        _ 250 ul of a 200 ug/ml Base Neutral Surrogate Solution

- If the samples is to be spiked for 625 analysis add:
        1000 ul of a 50 ug/ml matrix spiking solution.

- When a sample is to be extracted for Diazinon and Dursban the following must be added to each extraction vessel:
        1000 ul of a 1000 ng/ml Diazinon d10 solution
        1000 ul of a 1000 ng/ml Diazinon and Dursban for matrix spike if needed.

- Allow the samples to extract for 18 hrs.

- Concentrate the samples by closing the isolation valve that will allow solvent to be evaporated and recondensed into the main extraction chamber but will not allow any more solvent into the Kuderna-Danish/concentrator section of the extraction apparatus. Once most of the solvent has been removed water circulation can be stopped. The concentrator tube will come to ambient temperature. **Go to the sample clean-up steps if required.**

- Concentrate the extract to 1.0 ml by nitrogen blow-down using nitrogen supply taps on the extraction platform. This step is carried through at ambient temperature (no hot water running in water jacket)

STANDARD OPERATING PROCEDURE

GC/MS QA/QC for SIMM
(revised 1/1996)

1. GENERAL:

Fisons MD800 mass detector, GC 8060 gas chromatograph and AS 800 autosampler are used for analyzing semivolatile organics by selected ion monitoring (SIMM) method, Currently employed for PCB congeners, PAHs and Diazinon/Chlorpyrifos analysis.

The GCMS is hardware-tuned to meet the spectral criteria in SW-846 method 8270 for a 50 ng injection of decafluorotriphenylphosphine (DFTPP). Analysis is not started until these criteria are met. The criteria is demonstrated during each 12 hour period.

2. CALIBRATIONS:

.Five calibration points are analyzed to produce the instrument's initial calibration. The relative standard deviation of the five point's response factors must be less than 30% or the calibration is repeated or the mass spectrometer needs to be retuned and calibrated. The calibration is verified by running an independent second source standard. The standard must be within 30% of it's certified value. If the standard does not meet established criteria, appropriate corrective action is taken.

The midpoint standard is used as the continuing calibration check standard (CCCS) for each day or every 12 hour of data acquisition. The CCCS must also be within 30% of the initial calibration. When it falls out of calibration, a new calibration curve is run.

All solutions used for calibration are purchased through reputable vendors and have values certified to 99 +% accuracy. The certified values are traceable to NIST. When solutions are prepared, all preparations are logged into the standards logbook located in the GC/MS section.

3. REAGENTS:

Reagent water is generated from the BarnStead OganicPure system which should be demonstrated to be free from contamiation.

Primary standards are purchased from vendors who have QA programs traceable back to NIST; Keep them up to expiration date; Opened vials are kept up to one year.

Secondary standard solutions are the intermediate and are prepared from primary standards; Keep them up to one year or when they show signs of degradation.

Working standards are calibration and spiking solutions. They are normally good for one month.

Record all standard solution peparation with vial numbers in the standard log. Store all standard solutions at 4C in 1.8 ml vials with Teflon-lined caps.

4. BLANK, MS and MSD:

Analyze a method blank, a matrix spike and matrix spike duplicate for every 5% of number of samples or every batch of samples which ever is more frequent.

5. MAINTENANCE:

All instrument maintenance is performed according to manufacturer-recommended procedures. Maintenance calls and work orders are logged into the system's maintenance logbook.

6. RECORD-KEEPING:

It a part of the overall protocol to keep legible, complete and up-to-date all records of:

Sample collection and extraction and sample run-log in the SAMPLE WORK-UP binder.
Standard preparations in the STANDARDS binder.
Instrument tuning and mentainance in the SEMIVOLATILES MAINTENANCE binder.
Most current nitial calibration and second source standard check, MDL, method parameters and other references in the specific method's QA binder
Data accquisition and processing in data directory folders and file them in the data hardcopy cabinet.

For each data directory, backup to tape the following files

        raw data subdirectory: **masslab\data\yymmdd*.raw**
        most current acqsistion method files: **masslab\acqsmpdb\opp.***
        calibration curve file: **masslab\curvedb\opp????.cdb**
        current quantify method: **masslab\methdb\oppsim.mth**
        peak list files: **masslab\peakdb\pah????.pdb**

Name each group of files the same name as the sample list name (same as the day directory for raw data).

AQUA-Science Analytical Method for Diazinon and Chlorpyrifos

Appendix E

MILLIPORE

EnviroGard™ Diazinon Plate Kit
ENVR P00 32

Intended Use

The EnviroGard Diazinon Plate Kit is a quantitative laboratory test for the detection of diazinon residues in water.

Test Principles

The EnviroGard Diazinon Plate Kit is based on the use of polyclonal antibodies which bind either diazinon or a Diazinon-Enzyme Conjugate. These antibodies are immobilized to the walls of the test wells. When diazinon is present in the sample, it competes with Diazinon-Enzyme Conjugate for a limited number of antibody binding sites.

1. A sample containing diazinon is added to a test well, followed by Diazinon-Enzyme Conjugate. The Diazinon-Enzyme Conjugate competes with the diazinon for the antibody binding sites.

2. After this mixture is incubated for 1 hour, unbound molecules are washed away.

3. A clear solution of chromogenic Substrate is then added to the test well. In the presence of bound Diazinon-Enzyme Conjugate, the Substrate is converted to a compound which turns blue. One enzyme molecule can convert many Substrate molecules.

Since there are the same number of antibody binding sites on every test well, and each test well receives the same number of Diazinon-Enzyme Conjugate molecules, a sample which contains a low concentration of diazinon allows the antibody to bind many Diazinon-Enzyme Conjugate molecules. Therefore, a low concentration of diazinon will produce a dark blue solution.

Conversely, a high concentration of diazinon will allow fewer Diazinon-Enzyme Conjugate molecules to be bound by the antibodies, resulting in a lighter blue solution.

Note: Color is inversely proportional to diazinon concentration.

Darker color = lower concentration.
Lighter color = higher concentration.

Performance Characteristics

The EnviroGard Diazinon Plate Kit is very specific for diazinon and is essentially non-reactive, with the exception of pirimiphos-ethyl and pirimiphos-methyl, to closely-related organophosphate compounds and other pesticides. The following chart shows the concentration yielding 50% B_0* and the approximate concentration yielding 85% B_0, which is the Lower Limit of Detection (LLD), for a number of compounds. Concentrations are in parts per trillion (ppt) or parts per billion (ppb).

Compound	50% B_0*	LLD (85% B_0)
Diazinon	100 ppt	22 ppt
Diazoxon	900 ppt	200 ppt
Pirimiphos-ethyl	700 ppb	125 ppb
Pirimiphos-methyl	≥ 5000 ppb	900 ppb

* % Bo = average optical density (OD) of the calibrator or sample divided by the average OD of the negative control, multiplied by 100 (see "Calculate the Results".)

The following compounds are not detected at 1000 ppb:

Etrimfos	Fenitrothion	DDT
Fensulfothion	Fenchlorphos	Diuron
Bromophos	Bromophos-Methyl	
Tetrachlorvinphos	Parathion	
Methyl-Parathion	Paraoxon	
Chlorpyrifos	Chlorpyrifos-Methyl	
Azinphos-Methyl	Fenamiphos	
Methidathion	Dicapthon	
Temephos	Cythioate	
Atrazine	Simazine	
Chlorthal	Dieldrin	
Molinate	Diazinon Hydroxypyrimidine Metabolite	

Precautions

- Store all plate kit components at 4°C to 8°C (39°F to 46°F) when not in use.

- Do not freeze plate kit components or expose them to temperatures greater than 37°C (99°F).

- Allow all reagents to reach ambient temperature (18°C to 27°C or 64°F to 81°F) before beginning the test.

- Do not expose substrate to direct sunlight.

- Do not use plate kit components after the expiration date.

- Do not use reagents or test well strips from one plate kit with reagents or test well strips from a different plate kit.

- Do not dilute or adulterate test reagents or use samples not called for in the test procedure.

- Tightly re-cap the Diazinon Stock Solution after use to avoid evaporative losses.

- Use approved methodologies to confirm any positive results.

- Some solutes and particulates found in untreated ground or surface waters may affect the sensitivity level of this plate kit.

- Aqueous solutions of diazinon are affected by acidic conditions. Collect all samples and prepare all calibrators in glassware that has been rinsed free of all acidic detergent residues.

Materials Provided

Make sure you have the following items in your plate kit:

8 antibody-coated strips (12 wells each), in strip holder
1 vial of 100 nanogram/milliliter (ng/mL) Diazinon Stock Solution
1 vial of Diazinon-Enzyme Conjugate
1 vial of Substrate
1 vial of Stop Solution

Materials Required - Not Provided

You will also need these other items:

- 10 mL volumetric flask
- positive-displacement pipette which will measure 50 microliters (μL)
- disposable-tip pipette which will measure 100 μL
- pipette(s) which will measure 0.3, 1.0, 4.0 and 4.7 mL
- Milli-RO® or Milli-Q® water (or the equivalent) for calibrator preparation
- glass tubes or vials for calibrator preparation
- marking pen

- tape or Parafilm®
- timer (1 hour and 30 minutes)
- tap or distilled water for rinsing wells
- orbital shaker (optional)
- microtiter plate reader or strip reader
- calculator which performs linear regression (optional)
- microtiter plate washer (optional)
- a multi-channel pipette (optional)

Prepare The Calibrators

The EnviroGard Diazinon Plate Kit contains a 100 ng/mL (100 ppb) Stock Solution of Diazinon in methanol. *Do not use the stock solution directly in the assay.* This Stock Solution must be diluted in laboratory grade water in order to prepare 30, 100 and 500 ppt calibrators.

Note: Accurate pipetting of the Stock Solution and thorough mixing of the calibrator solutions are critical to the performance of this assay.

1. Be certain that the 100 ng/mL Diazinon Stock Solution is at room temperature. Gently swirl the vial to mix before pipetting.

2. Prepare the 500 ppt calibrator by pipetting 50 μL Diazinon Stock Solution into a 10 mL volumetric flask (use a positive-displacement pipette to measure the Stock Solution). Bring it to volume with Milli-Q or Milli-RO water (or the equivalent).

3. Prepare the 100 ppt calibrator by mixing 1.0 mL of the 500 ppt calibrator with 4.0 mL water.

4. Prepare the 30 ppt calibrator by mixing 0.3 mL of the 500 ppt calibrator with 4.7 mL water.

5. Milli-Q or Milli-RO water (or the equivalent) alone will be used as the Negative Control.

Note: These aqueous calibrators may be unstable and should be prepared fresh just prior to use.

Assay Procedure

The raised markings on the strip holder identify the well location while you add the reagents and samples. To add the calibrators, samples, Conjugate, Substrate, and Stop Solution, a 100 μL pipette must be used.

1. Two strips may be used to run the Negative Control , three calibrators , and eight samples in duplicate. For example:

Negative Control (C)
Calibrator 1 (C1) = 30 ppt
Calibrator 2 (C2) = 100 ppt
Calibrator 3 (C3) = 500 ppt
Samples (S1, S2, S3, etc.)

	1	2	3	4	5	6	7	8	9	10	11	12
A	C	C	C1	C1	C2	C2	C3	C3	S1	S1	S2	S2
B	S3	S3	S4	S4	S5	S5	S6	S6	S7	S7	S8	S8
C												
D												
E												
F												
G												
H												

Note: When you use fewer than eight strips, remove the unneeded strips and store them at 4°C to 8°C (39°F to 46°F) in the re-sealable plastic bag (with desiccant) provided.

2. Add 100 µL of Negative Control (C) and each calibrator (C1 to C3), and 100 µL of each sample (S1 to S8) to their respective wells, as shown above.

3. Using the same order of addition, add 100 µL of Diazinon-Enzyme Conjugate to each well.

Note: If you are running more than three strips, it is recommended that a multi-channel pipette be used in steps 2, 3, 7, and 9.

4. Thoroughly mix the contents of the wells by moving the strip holder in a rapid circular motion on the benchtop. Be careful not to spill the contents!

5. Cover the wells with tape or Parafilm to prevent evaporation and incubate at ambient temperature for 1 hour. During incubation, orbital mixing at 200 rpm is preferable, but not mandatory.

6. After incubation, carefully remove the covering and vigorously shake the contents of the wells into a sink. Flood the wells completely with cool running tap water, then shake to empty. Repeat this wash step five times. Invert the plate and tap out as much water as possible.

Alternatively, use a microtiter plate washer for the wash steps.

7. Add 100 µL of Substrate to each well, beginning with the negative control (C) and calibrators (C1 to C3), then the samples (S1 to S8).

8. Mix the contents of the wells, as in step 4. Cover the wells with new tape or Parafilm and incubate at ambient temperature for 30 minutes. During incubation, orbital mixing at 200 rpm is preferable, but not mandatory.

Warning: Stop Solution is 1 N hydrochloric acid. Handle carefully.

9. Add 100 µL of Stop Solution to each well and shake to mix thoroughly. This will turn the solution yellow.

Note: Read the plate as soon as possible. The color is unstable beyond 30 minutes.

Interpret The Results
Spectrophotometric Measurement and Analysis

1. Adjust the wavelength of your microtiter plate reader to 450 nanometers (nm). (If it has dual wavelength capability, use 600 or 650 nm as the "reference" wavelength.)

2. If the plate reader does not auto-zero on air, zero the instrument against 200 µL water in a blank well, then measure and record the optical density (OD) of each well's contents. Or, measure and record the OD in every well, then subtract the OD of the water blank from each of the readings.

3. If the microtiter plate reader you are using has data reduction capabilities, use a semi-log curve fit for the standard curve. You can also calculate the results manually as described in the next section.

Calculate the Results

1. After the wells have been read, average the OD of each set of calibrators and samples, and calculate the %B$_0$ as follows:

$$\%B_0 = \frac{\text{average OD of calibrator or sample}}{\text{average OD of negative control}} \times 100$$

The %B$_0$ calculation is used as a means of equalizing different runs of an assay. While the raw OD readings of negative controls, calibrators, and samples are likely to differ from run to run, the %B$_0$ relationship of calibrators and samples to the negative control should remain fairly constant.

NOTE: To ensure accurate results, you should meet the following guidelines for each of the three calibrators you are testing. The %CV [Coefficient of Variation = (standard deviation/mean) x 100] for the calibrator OD values should not exceed 15%.

2. Graph the %B$_0$ of each calibrator against its diazinon concentration on a semi-log scale (see *"Sample Calculations"*).

3. Determine the diazinon concentration of each sample by finding its %B$_0$ value and the corresponding concentration level on the graph.

4. Interpolation of sample concentration is only valid if the %B$_0$ of the sample falls within the range of the %B$_0$'s set by the calibrators. If the %B$_0$ of a sample is lower than that of the highest calibrator, dilute that sample with laboratory grade water so it falls on the standard curve when you run the assay again.

Sample Calculations

Well contents	OD	Average OD±SD**	%CV	%B$_0$	Diazinon conc. (ppt)
Negative Control	1.507 1.451	1.479 ± 0.040	2.68	100.0	N/A
30 ppt Calibrator	1.082 1.034	1.058 ± 0.034	3.21	72	N/A
100 ppt Calibrator	0.650 0.655	0.652 ± 0.004	0.54	44	N/A
500 ppt Calibrator	0.227 0.204	0.216 ± 0.016	7.55	15	N/A
Sample	0.859 0.807	0.833 ± 0.037	4.41	56	61

Actual values may vary; this data is for example purposes only.

_____
** standard deviation

Example

Diazinon Concentration (ppt)
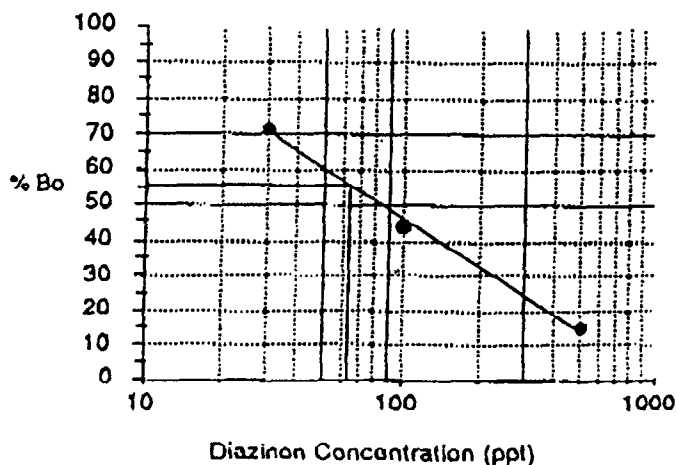
- Aqueous solutions of chlorpyrifos may stick to plastics and are destroyed by alkaline conditions. Therefore, collect all samples and prepare all calibrators in glassware that has been rinsed free of all alkaline detergent residues.

- Store chlorpyrifos samples and solutions in glass containers. Do not store them in plastic.

- Tightly recap the Chlorpyrifos Stock Solutions to prevent evaporative loss.

- Use approved methodologies to confirm results.

- Do not expose substrate to direct sunlight.

Materials Provided

Make sure you have the following items in your plate kit:

8 antibody-coated strips (12 wells each), in strip holder
1 vial of 1.0 part per million (ppm) Chlorpyrifos-ethyl Stock Solution
1 vial of 1.0 ppm Chlorpyrifos-methyl Stock Solution
1 vial of Chlorpyrifos-Enzyme Conjugate
1 vial of Substrate
1 vial of Stop Solution (Caution! 1.0N Hydrochloric Acid)
1 vial of 5X Diluent (**For Grain Extracts Only**)

Materials You Provide

You also need these items:

- 100 milliliter (mL) glass volumetric flask
- Positive-displacement pipette and tips that will measure 10 microliters (μL)
- Disposable-tip pipette and and disposable tips that will measure 100 μL
- Pipettes that will measure 0.5 mL, 2.0 mL, 3.0 mL, 5.0 mL, 7.0 mL, 8.0 mL, 9.5 mL and 10 mL.
- Milli-Q® or Milli-RO® water (or equivalent) for calibrator preparation
- Glass tubes or vials for calibrator preparation Caution: **Do not use plastic.**
- marking pen (indelible)
- tape or Parafilm®
- timer (1 hour and 30 minutes)
- tap or distilled water for rinsing wells
- microtiter plate reader or strip reader
- a multi-channel pipette (optional)
- microtiter plate washer (optional)
- orbital shaker (optional)
- calculator (optional)

Sample Collection and Storage

Chlorpyrifos breaks down under alkaline conditions. If your samples are not stored frozen or analyzed immediately after collection the samples should be buffered to a neutral or slightly acidic pH.

Prepare the Calibrators

The EnviroGard Chlorpyrifos Plate Kit contains a 1.0 ppm Stock Solution of chlorpyrifos-ethyl and a 1.0 ppm Stock Solution of chlorpyrifos-methyl. Both are in methanol/acetic acid. The kit can be calibrated with either chlorpyrifos-ethyl or chlorpyrifos-methyl.

CAUTION: Do not use these Stock Solutions directly in the assay.

The Stock Solutions must be diluted in laboratory-grade water. Use Milli-Q or Milli-RO water (or the equivalent) as a Negative Control in each assay.

Chlorpyrifos-ethyl Calibrators

1. Make sure the 1.0 ppm Chlorpyrifos-ethyl Stock Solution is at room temperature. Gently swirl the vial to mix before pipetting.

2. Prepare a 1.0 ppb calibrator by using a positive-displacement pipette to pipette 10 μL Chlorpyrifos-ethyl Stock Solution into 10 ml. Milli-Q or Milli-RO water (or the equivalent).

3. Prepare a 0.3 ppb calibrator by mixing 3.0 ml. of the 1.0 ppb calibrator with 7.0 mL of water.

4. Prepare a 0.05 ppb calibrator by mixing 0.5 ml. of the 1.0 ppb calibrator with 9.5 mL of water.

CAUTION: Accurate pipetting of the Chlorpyrifos-ethyl Stock Solution and thorough mixing of the calibrator solutions are critical to the performance of this assay. These aqueous calibrators are unstable and should be prepared immediately before use.

Chlorpyrifos-methyl Calibrators

1. Make sure the 1.0 ppm Chlorpyrifos-methyl Stock Solution is at room temperature. Gently swirl the vial to mix before pipetting.

2. Prepare a 0.1 ppb calibrator by using a positive-displacement pipette to pipette 10 μL Chlorpyrifos-methyl Stock Solution into 100 ml. of Milli-Q or Milli-RO water (or the equivalent) in a 100 mL volumetric flask.

3. Prepare a 0.05 ppb calibrator by mixing 5.0 mL of the 0.1 ppb calibrator with 5.0 mL of water.

MILLIPORE

EnviroGard™ Chlorpyrifos Plate Kit

ENVR P00 18

Intended Use

The EnviroGard Chlorpyrifos Plate Kit is a quantitative laboratory test for the detection of chlorpyrifos-ethyl or chlorpyrifos-methyl in water. The kit can be calibrated with chlorpyrifos-ethyl or chlorpyrifos-methyl. An attached application sheet for analysis of chlorpyrifos-methyl in grain extracts is included.

Test Principles

The EnviroGard Chlorpyrifos Plate Kit uses polyclonal antibodies which bind either chlorpyrifos or a chlorpyrifos-enzyme conjugate. These antibodies are immobilized to the walls of the test wells. Chlorpyrifos in the sample competes with the chlorpyrifos-enzyme conjugate for a limited number of antibody binding sites. In the assay procedure you will:

1. Add a sample containing chlorpyrifos to a test well, followed by chlorpyrifos-enzyme conjugate. The chlorpyrifos-enzyme conjugate competes with the chlorpyrifos for the antibody binding sites.

2. Wash away any unbound molecules after you incubate this mixture for 1 hour .

3. A clear solution of substrate is then added to the test well. In the presence of bound chlorpyrifos enzyme-conjugate, the substrate is converted to a compound which turns blue. One enzyme molecule can convert many substrate molecules.

Since the same number of antibody binding sites are available on every test well, and each test well receives the same number of chlorpyrifos-enzyme conjugate molecules, a sample which contains a low concentration of chlorpyrifos allows the antibody to bind many chlorpyrifos-enzyme conjugate molecules. The result is a dark blue solution.

Conversely, a high concentration of chlorpyrifos allows fewer chlorpyrifos-enzyme conjugate molecules to be bound by the antibodies, resulting in a lighter blue solution.

NOTE: Color is inversely proportional to chlorpyrifos concentration.

Darker color = lower concentration.
Lighter color = higher concentration.

Performance Characteristics

The EnviroGard Chlorpyrifos Plate Kit does not differentiate between chlorpyrifos and other closely related compounds, but detects their presence to differing degrees. The following chart shows the concentration yielding 50% B_0* and the approximate concentration of the lower limit of detection (LLD) for a number of compounds. Concentration is in parts per billion (ppb).

Compound	50% B_0	LLD
Chlorpyrifos-Ethyl	0.8	0.05
Chlorpyrifos-Methyl	0.05	0.02
3,5,6-Trichloro-2-Pyridinol	>1000	100
Azinphos	298	10
Azinphos-Methyl	715	82
Bromophos	3	0.5
Bromophos-Methyl	0.4	0.07
Fenchlorphos	0.6	0.01
Fenitrothion	450	40
Parathion-Methyl	522	50
Picloram	>1000	1000
Quinalfos	200	20
Triazophos	30	2
Triclopyr	48	3

%B_0 equals the average optical density (OD) of the calibrator or sample, divided by the average OD of the negative control, multiplied by 100 (see "Calculate the Results").

Precautions

• Store all plate kit components at 4°C to 8°C (39°F to 46°F) when not in use.

• Do not store test kit components for more than 8 hours at ambient temperatures (20°C to 37°C or 68°F to 99°F).

• Do not freeze plate kit components or expose them to temperatures greater than 37°C (99°F).

• Allow all reagents to reach ambient temperature (18°C to 27°C or 64°F to 81°F) before beginning the test.

• Do not use plate kit components after the expiration date.

• Do not use reagents or test well strips from one plate kit with reagents or test well strips from a different plate kit.

• Do not dilute or adulterate test reagents or use samples not called for in the test procedure.

4. Prepare a 0.02 ppb calibrator by mixing 2.0 mL of the 0.1 ppb calibrator with 8.0 mL of water.

CAUTION: Accurate pipetting of the Chlorpyrifos-methyl Stock Solution and thorough mixing of the calibrator solutions are critical to the performance of this assay. These aqueous calibrators are unstable and should be prepared immediately before use.

Assay Procedure for the Detection of Chlorpyrifos in Water

The raised markings on the strip holder identify the well location as you add the reagents and samples.

1. Two strips may be used to run the Negative Control, three Calibrators and eight samples in duplicate. For example (using Chlorpyrifos-ethyl calibrators):

   Negative Control (C) = Milli-Q or Milli-RO water
   Calibrator 1 (C1) = 0.05 ppb calibrator
   Calibrator 2 (C2) = 0.3 ppb calibrator
   Calibrator 3 (C3) = 1.0 ppb calibrator
   Samples (S1, S2, S3, S4, S5, S6, S7 and S8)

	1	2	3	4	5	6	7	8	9	10	11	12
A	C	C	C1	C1	C2	C2	C3	C3	S1	S1	S2	S2
B	S3	S3	S4	S4	S5	S5	S6	S6	S7	S7	S8	S8
C												
D												
E												
F												
G												
H												

NOTE: When you use fewer than eight strips, remove the unneeded strips and store them at 4°C to 8°C in the re-sealable plastic bag (with desiccant) provided.

2. Add 100 µL of Negative Control (C), each calibrator (C1 to C3), and each sample (S1 to S8) to their respective wells, as shown above.

3. Add 100 µL of Chlorpyrifos Enzyme Conjugate to each well in the same order of addition as calibrators and samples.

NOTE: If you are running more than three strips, you should use a multi-channel pipette in steps 2, 3, 7 and 9.

4. Thoroughly mix the contents of the wells by moving the strip holder in a rapid circular motion on the benchtop. Be careful not to spill the contents.

5. Cover the wells with tape or Parafilm to prevent evaporation and incubate at ambient temperature for 1 hour. During incubation, orbital mixing at 200 revolutions per minute (rpm) is recommended, but not mandatory.

6. After incubation, carefully remove the covering and vigorously shake the contents of the wells into a sink. Flood the wells completely with cool running tap or distilled water, then shake to empty. Repeat this wash step five times. Invert the plate and tap out as much water as possible. Alternatively, use a microtiter plate washer for the wash steps.

7. Add 100 µL of Substrate to each well, beginning with the Negative Control (C) and calibrators (C1 to C3), then the samples (S1 to S8).

8. Cover the wells with new tape or Parafilm and incubate at ambient temperature for 30 minutes. During incubation, orbital mixing at 200 rpm is preferable, but not mandatory.

WARNING: Stop Solution is 1N Hydrochloric acid. Handle carefully.

9. Add 100 µL of Stop Solution to each well and mix thoroughly. The solution will turn yellow.

NOTE: Read the plate within 30 minutes of adding the Stop Solution.

INTERPRET THE RESULTS

Spectrophotometric Measurement and Analysis

1. Adjust the wavelength of your microtiter plate reader to 450 nanometers (nm). (If it has dual wavelength capability, use 600 or 650 nm as the "reference" wavelength.)

2. If the plate reader does not auto-zero on air, zero it against 200 µL water in a blank well, then measure & record the optical density (OD) of each well's contents. Or, measure & record the OD in every well, then subtract the OD of the water blank from each of the readings.

3. If the microtiter plate reader you are using has data reduction capabilities, use a semi-log curve fit for the standard curve. You can also calculate the results manually as described in the next section.

Calculate the Results

1. After you read all of the wells, average the OD of each set of calibrators and samples, and calculate the %B_0 as follows:

$$\%B_o = \frac{\text{average OD of calibrator or sample}}{\text{average OD of negative control}} \times 100$$

The $\%B_O$ calculation is used as a means of equalizing different runs of an assay. While the raw OD readings of negative controls, calibrators, and samples are likely to differ from run to run, the $\%Bo$ relationship of calibrators and samples to the Negative Control should remain fairly constant.

NOTE: To ensure accurate results, you should meet the following guidelines for the calibrators you run. The $\%CV$ [Coefficient of Variation = (standard deviation/mean) x 100] for the calibrator OD values shouldn't exceed 15%.

Chlorpyrifos-ethyl Concentration (ppb) in Water	$\%B_o$ Range
0.05	78-96
0.3	35-63
1.0	12-25

Chlorpyrifos-methyl Concentration (ppb) in Water	$\%B_o$ Range
0.02	76-89
0.05	53-70
0.1	29-47

2. Graph the $\%B_O$ of each Calibrator against its Chlorpyrifos concentration on a semi-log scale (see "Sample Calculations").

3. Determine the Chlorpyrifos concentration of each sample by finding its $\%B_O$ value and the corresponding concentration level on the graph.

4. Interpolation of sample concentration is only valid if the $\%B_O$ of the sample falls within the range of the $\%B_O$'s set by the calibrators. If the $\%B_O$ of a sample is lower than that of the highest calibrator, dilute that sample so it falls on the standard curve when you run the assay again, then multiply by the dilution factor.

Sample Calculations (using chlorpyrifos-ethyl calibrators)

Well contents	OD	Average OD±SD*	%CV	$\%B_O$	Chlorpyrifos-ethyl conc. (ppb)
Negative Control	1.762 1.700	1.731±0.044	2.5	100	N/A
0.05 ppb Calibrator	1.507 1.443	1.475±0.045	3.1	85	N/A
0.3 ppb Calibrator	0.895 0.839	0.867±0.040	4.6	50	N/A
1.0 ppb Calibrator	0.301 0.292	0.297±0.006	2.1	17	N/A
Sample	1.354 1.299	1.327±0.039	2.9	77	0.078

NOTE: Actual values may vary; this data is for demonstration purposes only.

* standard deviation

Example (using chlorpyrifos-ethyl calibrators):

Chlorpyrifos-ethyl Calibrators (ppb)
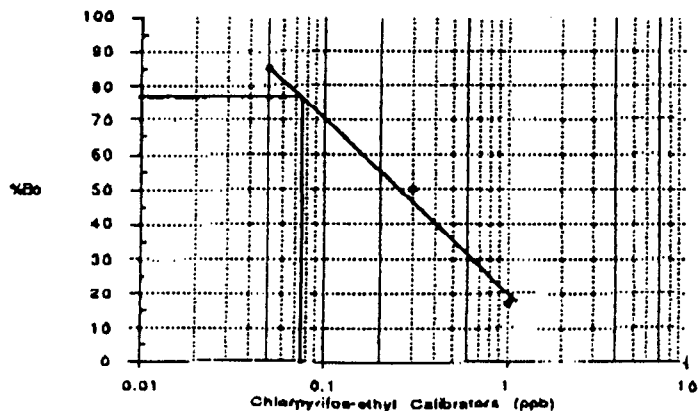
Detection of Chlorpyrifos-methyl in Grain
(For use with the EnviroGard™ Chlorpyrifos Plate Kit)

Sample Extraction Procedure

Before you can analyze chlorpyrifos-methyl in grain, you must extract it from the grain using methanol. Extracts do not require filtration before use. Note that other solvents may interfere with the test, and produce erroneous results. Extraction requires a minimum of 20 grams (g) of grain for good results. Sample size also depends on the equipment available and application of the test (silo distribution studies, shipping samples, etc.).

You may use one of the extraction methods below, depending on whether you use whole or ground grain, the time you allot for sample extraction, and the detection level of chlorpyrifos-methyl you require.

Extraction Using Whole Grain

Overnight soaking of whole grain is the simplest method and is suitable when results are not required immediately. Soak whole grain in 2.5 mL neat methanol/gram of grain, swirl for 1-2 minutes by hand, allow to sit for 16-48 hours, swirl again then analyze. Pesticide extraction is complete after 48 hours but is 90% complete after 16 hours.

You can also extract pesticide with over 80% efficiency from whole grain by blending the grain for 1 minute with 2.5 mL neat methanol/g of grain using a high-speed laboratory blender approved for use with solvents.

Extraction Using Ground Grain

You can use grain that has previously been ground. Alternatively, you may use a domestic coffee grinder or other suitable grinder for grinding the grain.

You can extract pesticide quantitatively (over 90% extraction efficiency) using a high-speed laboratory blender approved for use with organic solvents or a high-speed probe or blade homogenizer by blending for 1 minute in 2.5 mL neat methanol/gram ground grain. Allow the extract to settle and remove a sample of the supernatant for analysis.

If results are not required immediately, overnight soaking is the simplest method. Soak ground grain in 2.5 mL neat methanol/gram of ground grain, swirl for 1 - 2 minutes by hand, allow to sit for 16 to 48 hours, swirl again, then analyze the supernatant.

Prepare Working Diluent Buffer

Add the entire container of 5X Grain Diluent to 120 mL of purified water. Mix thoroughly and store at 4°C when not in use. This is used to dilute calibrators and samples in the steps following.

Preparation of Calibrators

1. Prepare calibrators equivalent to 0 ppm, 0.5 ppm, 2.5 ppm and 7.5 ppm in grain by diluting the 1 ppm Chlorpyrifos-methyl Stock Solution as follows*:

calibrator level	mL 1 ppm stock	mL methanol
7.5 ppm	0.3 mL	9.7 mL

   Use this 7.5 ppm calibrator to prepare these additional calibrators:

calibrator level	mL 7.5 ppm	mL methanol
0.5 ppm	0.67 mL	9.33 mL
2.5 ppm	3.33 mL	6.67 mL

* The dilutions of the 1 ppm Chlorpyrifos-methyl Stock Solution you are instructed to make actually result in calibrators of 0.3, 0.1 and 0.02 ppb. They correspond to 7.5, 2.5 and 0.5 ppm in grain, however, because of the dilution of grain samples before running the assay. (Example: 0.3 ppb x 2.5 x 100 x 100 = 7.5 ppm)

The negative control is neat methanol.

2. Dilute the negative control and calibrators by adding 10 μL of negative control or calibrator to 1 mL Working Diluent Buffer. Mix thoroughly and use within one hour.

Preparation of Samples

1. Dilute your samples by adding 10 μL of extract to 1 mL of methanol.

2. Further dilute your samples by adding 10 μL of the sample methanol solution from the above step to 1 mL of Working Diluent Buffer. Mix thoroughly and use within one hour.

Assay Procedure

Continue with the "Assay Procedure for the Detection of Chlorpyrifos in Water" (p. 3).

Dow-Elanco Analytical Method for Diazinon and Chlorpyrifos

I.   Determination of Recovery of Chlorpyrifos and Diazinon from Water

    1. Preparation of Recovery Samples

        a. Pipet 100.0-mL portions of the control water sample into a series of 4-oz bottles.

        b. For preparing fortified samples, use some of the samples as controls and fortify the remaining samples by adding 100-μL aliquots of the appropriate spiking solutions (prepared in acetone) to obtain concentrations ranging from 0.010 to 1.00 ng/mL. A reagent blank, containing no sample, should be carried through the method with the samples.

        c. Add 10 g of sodium chloride and 10 mL of hexane to the sample bottle, cap the bottle with a PTFE-lined cap, and shake the sample for 20 minutes on a reciprocating shaker at approximately 180 excursions/minute.

        d. Centrifuge the sample bottle for 5 minutes at 2100 rpm.

        e. Transfer the hexane (top) layer into a clean 40-mL vial. (In transferring the hexane layer, it is important not to remove any water from the lower layer.)

        f. Add an additional 10 mL of hexane to the sample bottle. Cap the bottle with a PTFE-lined cap, and shake the sample for 20 minutes on a reciprocating shaker at approximately 180 excursions/minute.

        g. Centrifuge the sample bottle for 5 minutes at 2100 rpm.

        h. Combine the hexane layer from Step I.1.g. with the hexane extract from Step I.1.e. (In transferring the hexane layer, it is important not to remove any water from the lower layer.)

        i. Add 1.0 mL of the internal standard solution (100 ng/mL butathiofos in hexane) to the sample vial.

        j. Concentrate the solution from Step I.1.i. to less than 4 mL (but not to dryness) using an N-Evap evaporator. (Note L.2.)

        k. Transfer the hexane from Step I.1.j. to a clean 4-mL vial.

        l. Rinse the 40-mL vial with 1 mL of hexane and transfer the rinse to the 4-mL vial.

        m. Concentrate the solution from Step I.1.l. to less than 0.5 mL (but not to dryness) using an N-Evap evaporator set at a water bath temperature of 40 °C and a nitrogen flow rate of approximately 200 mL/min.

n. Adjust the volume in the sample vial to 0.5 mL with hexane and firmly seal with a PTFE-lined cap. Vortex the sample for 5-10 seconds, and then sonicate the sample for 5-10 seconds.

o. Transfer the sample to a 2-mL autosampler vial containing a limited-volume insert and seal the vial with a cap.

p. Analyze the calibration standards from Section G.1.f. and samples by capillary gas chromatography/mass spectrometry as described in Section H.2. Determine the suitability of the chromatographic system using the following performance criteria:

   (1) Standard curve linearity: Determine that the correlation coefficient equals or exceeds 0.995 for the least squares equation which describes the detector response as a function of standard curve concentration. If power regression is used, the power exponent should be between 0.90-1.10.

   (2) Peak resolution: Visually determine that sufficient resolution has been achieved for the analytes and internal standard relative to background interferences.

   (3) Appearance of chromatograms: Visually determine that the chromatograms resemble those shown in Figures 8-13 with respect to peak response, baseline noise, and background interference. Visually determine that a minimum signal-to-noise ratio of 10:1 has been attained for each analyte in the 5.0-ng/mL calibration standard (equivalent to 0.025 ng/mL in water samples).

H.   Gas Chromatography/Mass Spectrometry

1. Column

Install the splitless column inlet sleeve and capillary column in the split/splitless
injection port of the GC/MSD following the manufacturer's recommended procedures.

2. Typical Operating Conditions

Instrumentation:	Hewlett-Packard Model 5890A gas chromatograph
	Hewlett-Packard Model 7673 automatic injector
	Hewlett-Packard Model 5972A mass selective detector
	Hewlett-Packard Model G1034C data system software
Column:	J & W Scientific fused silica capillary
	Durabond-17 liquid phase
	10 m x 0.18 mm i.d.
	0.3-μm film thickness

Temperatures:

Column	70 °C for 1.0 min
	70 °C to 220 °C at 10 °C/min
	220 °C to 280 °C at 20 °C/min
	280 °C for 1.0 min
Injector	260 °C
Interface	280 °C

Carrier Gas:	helium
Head Pressure	35 kPa
Linear Velocity	approximately 45 cm/s
	(vacuum compensation 'On')
Injection Mode:	splitless
Purge Delay	0.9 min
Splitter Flow	50 mL/min
Septum Purge	1.0 mL/min
Injection Volume:	3 μL

Detector: electron impact selected ion monitoring

   Calibration Program   maximum sensitivity autotune; usertune
   Electron Multiplier   2000 volts (~ 50 volts below autotune)

Ions Monitored:

   Chlorpyrifos m/z 314 (quantitation)
                                      m/z 316 (confirmation)

   Diazinon m/z 304 (quantitation)
                                       m/z 276 (confirmation)

   Butathiofos m/z 289 (internal standard)

Dwell Time: 75 ms

Mass spectra of chlorpyrifos, diazinon, and butathiofos are shown in Figures 3-5.

3. <u>Calibration Curves</u>

Typical calibration curves for the determination of chlorpyrifos and diazinon are shown in Figures 6-7.

4. <u>Typical Chromatograms</u>

Typical chromatograms of a standard, control sample, and a 0.025-ng/mL recovery sample for the determination of chlorpyrifos and diazinon in water are illustrated in Figures 8-13, respectively.

Ciba-Geigy/Novartis Analytical Method for Diazinon and Chlorpyrifos

OUTLINES FOR ANALYZING DIAZINON AND METABOLITES IN WATER

PROCEDURAL RECOVERY

	Percent Recovered			
	Diazinon		Diazoxon	CGA-14128
	NP	MSD		
Deionized water (2) + 1.0 ppb Diazinon Mix[1]	102	92	104	102
Deionized water (2) + 0.10 ppb Diazinon Mix[1]	76	70	75	83

Diazinon: O,O-Diethyl-O-(2-isopropyl-6-methyl-4-pyrimidinyl)phosphorothioate   MW=304

Diazoxon: O,O-Diethyl-O-(2-isopropyl-6-methyl-4-pyrimidinyl)phosphorate   mW=288

CGA-14128:   O,O-Diethyl-O-[2-(2-hydroxy-2-isopropyl)-6-methyl-4-pyrimidinyl)phosphorothioate   mW=320

Comments: Results are not corrected for control values.
            Results are corrected for procedural recoveries <100%.

[1]Diazinon mix contains diazinon, diazoxon, CGA-14128.

Method of Analysis:

Extraction: 500 gram sample (500 ml) was partitioned 3x100 ml with methylene chloride.
                     The organic phase was dried through a small pad of anhydrous sodium sulfate.
                     Rinse the sodium sulphate with 25 ml of methylene chloride.

Detection: Samples were dissolved in an appropriate volume of acetone for analysis by
                     Capillary G.C. with NP detection. Diazinon residues were confirmed using
                     GC/MSD at electron impact mode and using SIM at M/Z = 137.1 and a qualifier
                     ion at M/Z = 179.1.

Column: GC/NPD: DB-1701 (30m with 0.32 mm i.d.) with a 0.25 micron film thickness.
                     GC/MSD: MS-5 (30m x 0.25 mm i.d.) with a 0.25 micron film thickness.

0259201 (CL-DOC.RTR.RS-WM) mas/lca:11/30/92

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DETERMINATION OF DIAZINON, DIAZOXON, AND CGA-14128 RESIDUES IN
CROPS, CROP FRACTIONS AND ANIMAL TISSUES USING GAS CHROMATOGRAPHY

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Table III.
Typical Standardization Data for Organophosphorous Standards[1]

PEAK HEIGHTS (integration events)

Standards (ng)	Diazinon	Diazoxon	CGA-14128
0.010	2992	2331	1466
0.020	4853	3789	2526
0.050	15338	11734	6570
0.100	30566	21635	13721
0.200	54849	41667	23679

Correlation			
coefficient:	0.9975	0.9991	0.9966
Intercept (events):	713	430	592
Slope (events/ng):	276403	207912	118427

[1] DB-1701 column with FPD detector (see Table I for conditions)

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Figure 1
CHEMICAL STRUCTURES

1) Diazinon (G-24480), O,O-diethyl-O-(2-isopropyl-6-methyl-4-pyrimidinyl) phosphorothioate

2) CGA-14128, O,O-diethyl-O-(2-[2-hydroxy-2-isopropyl]-6-methyl-4-pyrimidinyl)phosphorothioate

3) Diazoxon (G-24576), O,O-diethyl-O-(2-isopropyl-6-methyl-4-pyrimidinyl) phosphorate

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DETERMINATION OF DIAZINON, DIAZOXON, AND CGA-14128 RESIDUES IN CROPS, CROP FRACTIONS AND ANIMAL TISSUES USING GAS CHROMATOGRAPHY

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Table I.
Gas Chromatography Conditions

Instruments: Hewlett-Packard Models 5880 and 5890

	DB1701-FPD	DB1701-N/P	2%DEGS-FPD
Column Dimensions:	30m x 0.32mm	30m x 0.32mm	2mm x 4ft.
Film Thickness:	0.25 µ	0.25 µ	-
Carrier Gas:	He	He	He
Carrier Gas (ml/min):	4.0	4.0	40
Initial Column Temp.(°C):	60°	60°	172°
Temperature Program (°C):	60°, 1 min 60° – 195°, 30°/min	60°, 1 min 60° – 195°, 30°/min	isothermal[1]
Injector Temp.(°C):	200°	200°	180°
Detector Temp.(°C):	200°	275°	200°
Helium (makeup) flow:	36 ml/min	26 ml/min	--
Hydrogen flow:	75 ml/min	4 ml/min	75 ml/min
Air flow:	100 ml/min	115 ml/min	100 ml/min
Detector Sensitivity:	Table VIII	Table VIII	Table VIII
Injection Volume:	2 µl[2]	2 µl[2]	5 µl
Typical Retention (min):			
Diazinon:	9.1	9.	0.91
Diazoxon:	9.4	10.	1.36
CGA-14128:	11.9	12.	2.27
Attenuation:	2[3]	2[3]	2[3]

[1] Temp. adjusted for chlorpyrifos to give 2.5 min ret. time
[2] Splitless injection (1 min. purge time delay)

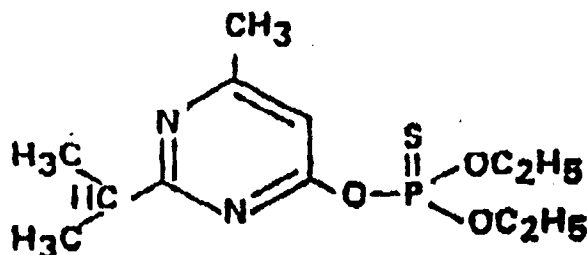CIBA-GEIGY CORPORATION
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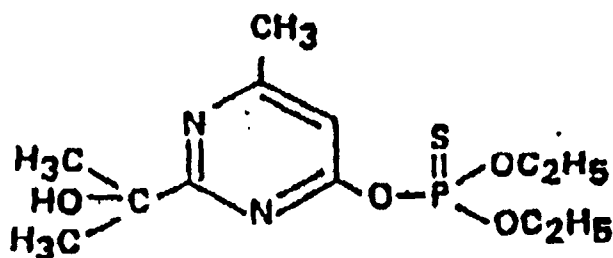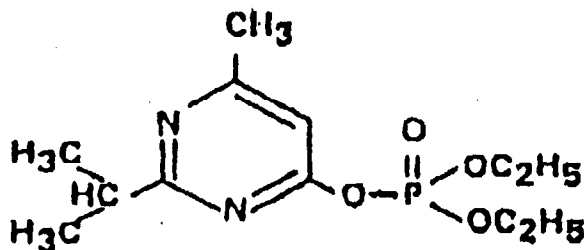
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Table II.
Gas Chromatography/Mass Spectrometry Conditions
(Confirmation of Diazinon Residues)

Instruments:
Gas Chromatograph: Hewlett-Packard Model 5890
Mass Selective Detector: Hewlett-Packard Model 5970

Column: DB1701
Column Dimensions: 30m x 0.32mm
Film Thickness: 0.25 µ

Carrier Gas: He
Carrier Gas (ml/min):   4.0
Initial Column Temp.(°C):   60°

Temperature Program (°C):   60°, 1 min
                                60° - 195°,
                                30°/min

Injector Temp.(°C):   250°
Transfer Temp.(°C):   260°
Source Temp.(°C):   300°

Injection Volume: 2 µl
(manual, splitless):   1 minute purge time delay

Typical Retention (min):
             Diazinon: 9.4

Electron Multiplier Voltage: 2000v

Recommended amu for
Selected Ion Monitoring[1]: 137, 179, 304

[1] 100 msec dwell time (See Figure 50 for typical mass spectrum)

Appendix F: Tabulated Influent, Residential, Commercial, and POTW Results

Table F1: Recovery-adjusted and Raw (in parenthesis) Daily Diazinon and Chlorpyrifos Concentrations in CCCSD Influent Samples

Date Sampled	Diazinon (ng/L)	Chlorpyrifos (ng/L)
6/22/96	940 (610)	150 (100)
6/25/96	103 (79)	110 (71)
6/29/96	350 (340)	160 (120)
7/2/96	320 (310)	160 (120)
7/9/96	380 (260)	170 (110)
7/10/96	230 (160)	120 (78)
7/11/96	300 (220)	160 (98)
7/12/96	480 (350)	160 (98)
7/13/96	290 (210)	160 (100)
7/14/96	320 (240)	200 (120)
7/15/96	310 (230)	200 (120)
7/16/96	150 (110)	160 (96)
7/20/96	270 (210)	130 (73)
7/23/96	240 (190)	200 (110)
7/27/96	690 (450)	330 (200)
7/30/96	300 (240)	160 (110)
8/4/96	280 (180)	200 (120)
8/5/96	190 (120)	230 (120)
8/6/96	750 (470)	210 (110)
8/7/96	530 (330)	180 (94)
8/8/96	130 (130)	210 (200)
8/9/96	150 (160)	190 (180)
8/10/96	180 (190)	140 (130)
8/11/96	210 (220)	190 (180)
8/13/96	150 (110)	190 (130)
8/17/96	310 (330)	240 (210)
8/20/96	170 (180)	150 (130)
8/27/96	270 (330)	98 (95)
8/31/96	290 (190)	210 (120)
9/1/96	640 (420)	190 (110)
9/2/96	340 (220)	600 (340)
9/3/96	230 (150)	250 (140)
9/4/96	180 (120)	280 (160)
9/5/96	140 (93)	190 (110)
9/6/96	150 (100)	130 (76)
9/7/96	400 (260)	220 (130)
9/10/96	400 (260)	270 (160)

Table F2: Recovery-adjusted Residential Diazinon and Chlorpyrifos Concentrations (ng/L) and Treated Data (with the robust probability plotting method fill-in values for results reported as none-detected)

Area #	Reported (raw) Concentrations		Recovery-adjusted Concentrations (with ND fill-in)	
	Diazinon (ng/L)	Chlorpyrifos (ng/L)	Diazinon (ng/L)	Chlorpyrifos (ng/L)
R01	110	270	160	410
R01	160	81	230	120
R01	3100	110	4300	180
R01	350	720	480	1200
R01	180	ND	240	*49*
R01	620	290	830	490
R01	110	700	150	1200
R02	94	ND	140	*59*
R02	1500	100	2200	150
R02	460	53	630	86
R02	89	56	120	91
R02	85	64	110	110
R02	100	70	140	120
R02	130	100	180	170
R03	ND	ND	*12*	*65*
R03	ND	ND	*48*	*22*
R03	ND	56	*41*	91
R03	720	ND	990	*22*
R03	ND	73	*34*	120
R03	60	ND	81	*54*
R03	ND	85	*20*	140
R04	66	ND	96	*39*
R04	ND	76	*27*	120
R04	89	96	120	160
R04	150	ND	210	*30*
R04	83	ND	110	*44*
R04	80	ND	110	*35*
R04	68	230	92	390
R05	83	100	120	150
R05	120	100	180	150
R05	560	120	770	190
R05	370	200	510	320
R05	360	74	490	120
R05	170	74	230	120
R05	73	ND	98	12

Table F3: Recovery-adjusted and Raw (in parenthesis) Daily Concentrations of Diazinon and Chlorpyrifos in Commercial Samples

Site Number	Date Sampled	Diazinon (ng/L)	Chlorpyrifos (ng/L)
C06	8/8/96	<48 (<50)	1400 (1300)
C06	8/9/96	<48 (<50)	1300 (1200)
C07	7/18/96	370 (280)	1400 (770)
C07	7/19/96	<65 (<50)	1200 (680)
C07	7/20/96	84 (64)	720 (400)
C08	9/5/96	350 (230)	38000 (22000)
C08	9/6/96	350 (230)	5500 (3200)
C08	9/8/96	76 (50)	12000 (6800)
C09	8/1/96	<78 (<50)	<81 (<50)
C09	8/2/96	<78 (<50)	<81 (<50)
C09	8/3/96	<78 (<50)	<81 (<50)
C10	8/22/96	1000 (1100)	<56 (<50)
C11*	6/20/96	13000 (8700)	3400 (2300)
C11	7/25/96	14000 (8900)	3600 (2200)
C11	7/26/96	13000 (8700)	960 (580)
C11	7/27/96	20000 (13000)	1000 (620)
C12*	6/20/96	<77 (<50)	<74 (<50)
C12	8/15/96	73 (79)	<57 (<50)
C12	8/16/96	<46 (<50)	<57 (<50)
C12	8/17/96	<46 (<50)	<57 (<50)
C13	8/14/96	130 (94)	91 (62)
C14	8/26/96	<46 (<50)	61 (54)
C14	8/27/96	<41 (<50)	<52 (<50)
C14	8/28/96	760 (920)	950 (920)
C15	7/29/96	<63 (<50)	300 (210)
C15	7/30/96	<63 (<50)	80 (56)
C15	7/31/96	<63 (<50)	230 (160)
C16	7/23/96	1100 (910)	350 (200)
C16	7/24/96	630 (410)	310 (190)
C17	8/19/96	93 (100)	920 (810)
C17	8/20/96	<46 (<50)	650 (570)
C17	8/22/96	54 (59)	200 (180)

* Grab sample not associated with flow measurements.

Table F4: Recovery-adjusted and Raw (in parenthesis) Daily Diazinon and Chlorpyrifos Concentrations in Influent Samples of CCCSD, USD, and RWQCP.

	Diazinon (ng/L)				Chlorpyrifos (ng/L)			
Date Sampled	CCCSD	USD	RWQCP	RWQCP (recycle)	CCCSD	USD	RWQCP	RWQCP (recycle)
8/5/96	190 (120)	180 (110)	190 (120)*	1100 (670)	230 (120)	290 (150)	150 (77*)	230 (120)
8/6/96	750 (470)	530 (330)	150 (91)	<79	210 (110)	290 (150)	120 (60)	<97
8/7/96	530 (330)	150 (93)	240 (150)	<79	180 (94)	330 (170)	130 (65)	<97
8/8/96	130 (130)	91 (95)	120 (120)	<48	210 (200)	130 (120)	83 (79)	<52
8/9/96	150 (160)	200 (210)	66 (69)	<48	190 (180)	180 (170)	<52	<52
8/10/96	180 (190)	170 (180)	150 (110)	<69	140 (130)	160 (150)	110 (74)	<73
8/11/96	210 (220)	350 (360)	110 (82)	<69	190 (180)	210 (200)	130 (90)	<73

* Values omitted from calculations since contamination from recycle flow was suspected.